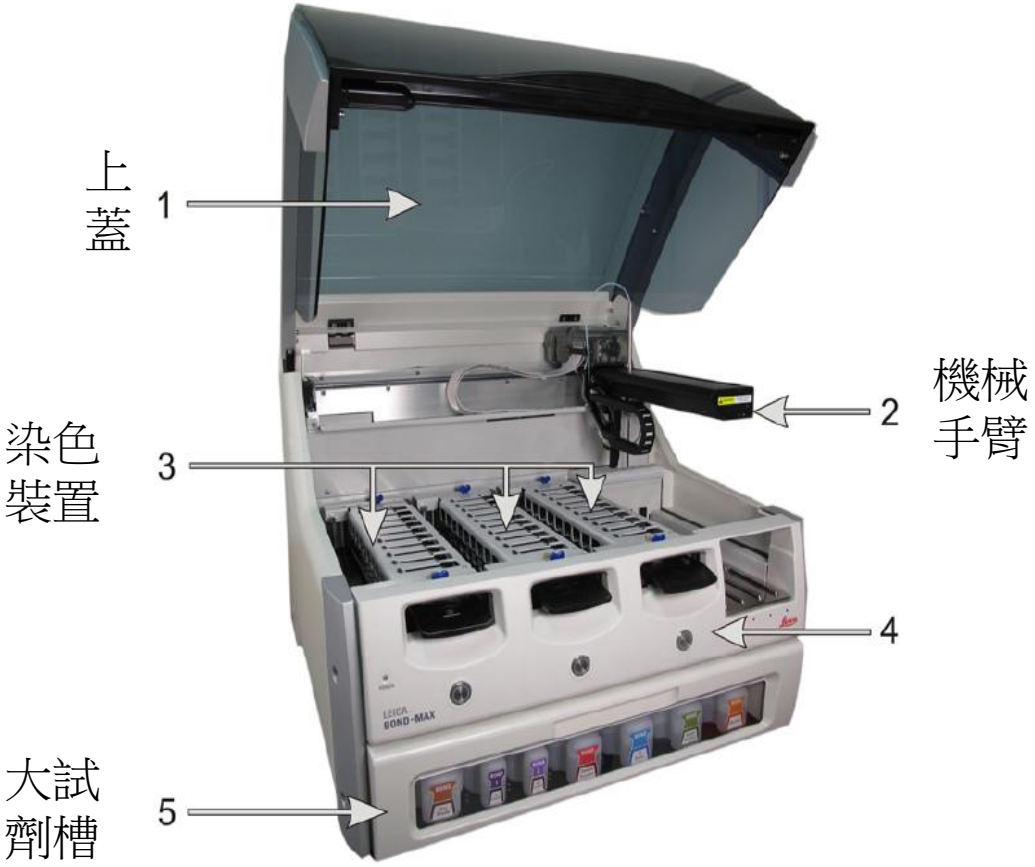
Leica Bond-Max免疫染色機介紹及應用

戴宇霆

龐德生技有限公司

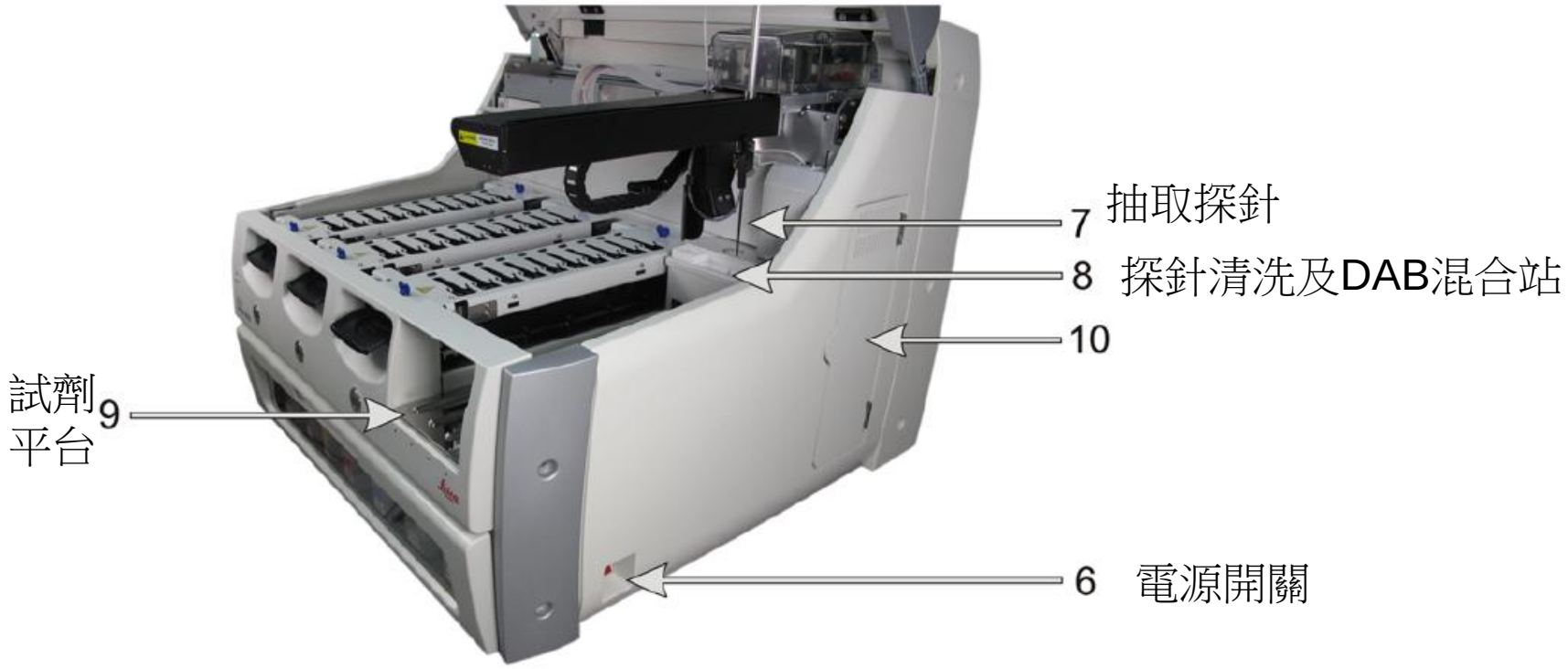
Bond™ - Continuous Batch Processing

Hardware 1



Note: A level sensor is connected to all bulk reagent containers to alert users when reagent levels are low

Hardware 2



Efficient Use Of Fluids
Efficient Use of Space

圖 18: BOND--MAX 外部廢液容器

工作站	容器	容量 (L)	色彩	試劑
1	有害廢液	2	棕色	有害廢液
2	ER1	1	紫色	BOND Epitope Retrieval Solution 1*
3	ER2	1	淡紫色	BOND Epitope Retrieval Solution 2*
4	脫蠟溶液	2	紅色	BOND Dewax Solution*
5	去離子水	2	藍色	去離子水
6	清洗緩衝液	2	綠色	BOND Wash Solution*
7	乙醇	2	橘色	酒精（試劑級）

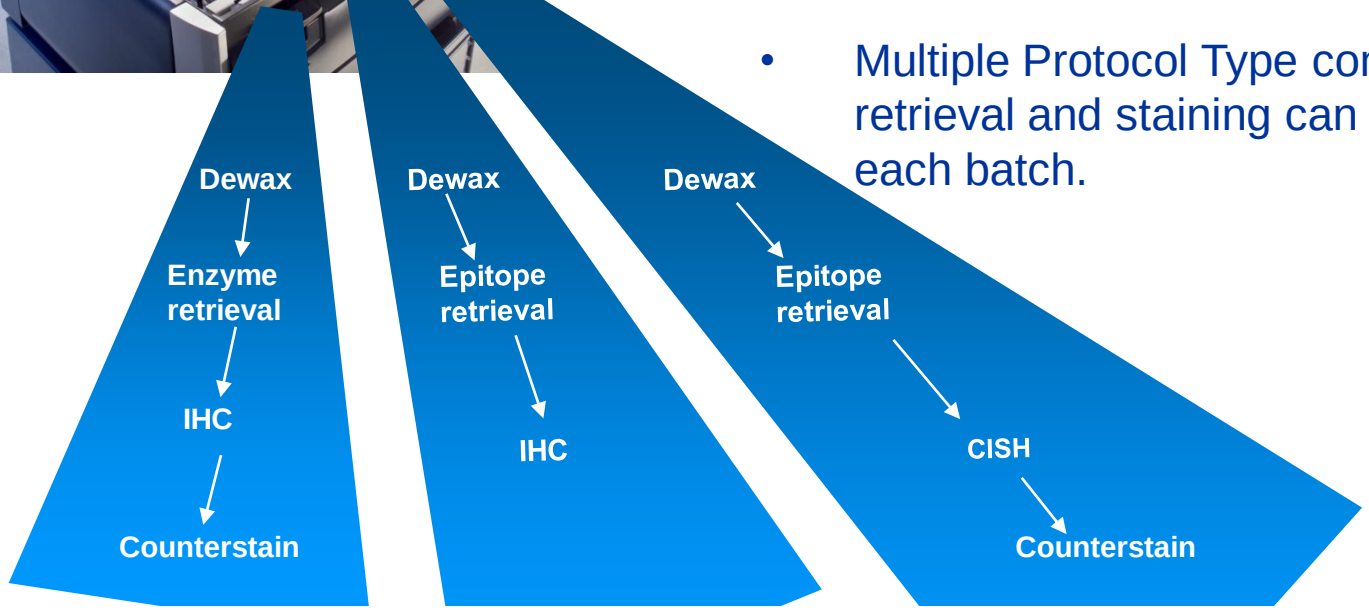
Note: A level sensor is connected to all bulk reagent containers to alert users when reagent levels are low

Leica
MICROSYSTEMS

Patient to
Pathologist

Bond™ Continuous Batch Processing

- Up to **3** Multiple Batches of **10 slides** on a single processing module
- Up to 10 different primary antibodies per batch
- Multiple Protocol Type combinations of dewax, retrieval and staining can be included within each batch.



Batch 1

10 Slide Capacity

Batch 2

10 Slide Capacity

Batch 3

10 Slide Capacity

total automation

press start then walk away – simple

IHC baking → dewax → epitope retrieval → staining → counterstaining

ISH baking → dewax → hybridisation → staining → counterstaining

Patient to Pathologist

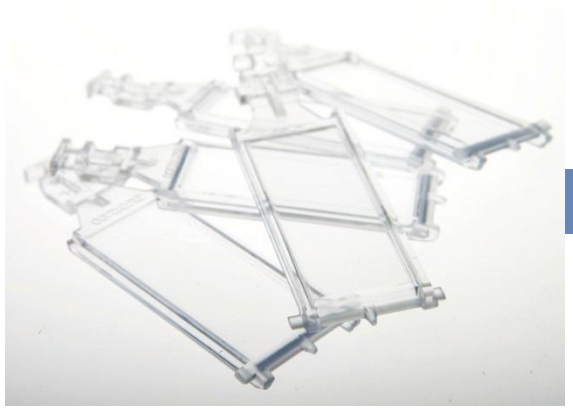
covertile technology

quality results

- Gentle reagent application
- Uniform reagent coverage on tissue
- Prevention of evaporation

tissue stays intact

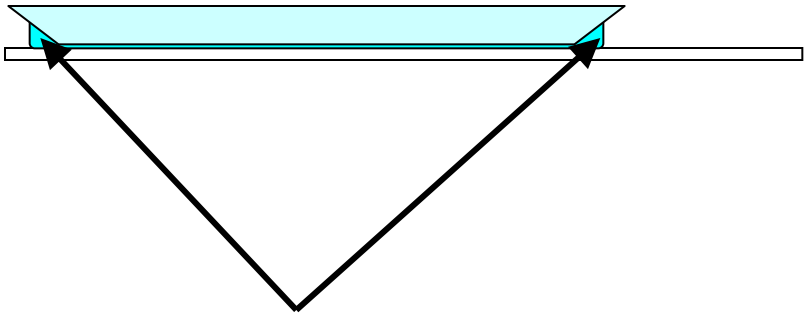
- No lifting
- Works on frozen sections
- Works on difficult tissue (bone marrow, fatty breast etc)



covertile technology
- controlled flow – no squirting
- no carryover of reagents
- no exposure to air between reagents

Bond™ Covertile Technology – "Reagent Reservoir"

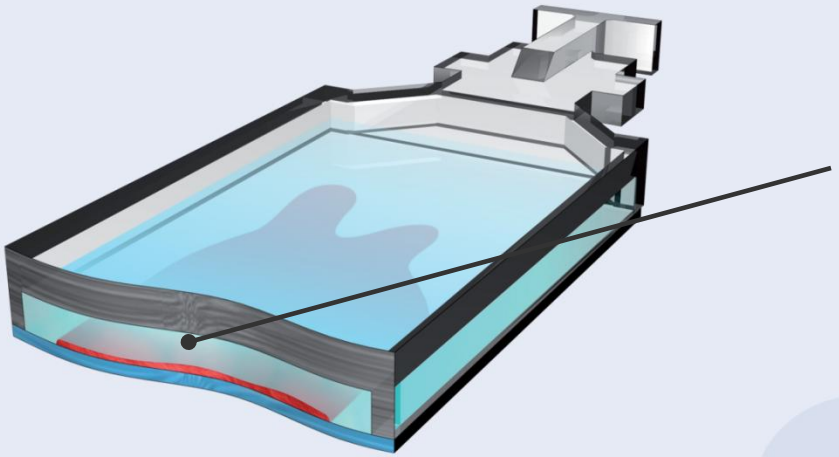
- During retrieval the cover tile moves onto the slide removing the path to the vacuum port and enabling a reagent reservoir to be formed at each end of the cover tile



Reagent Reservoir formed

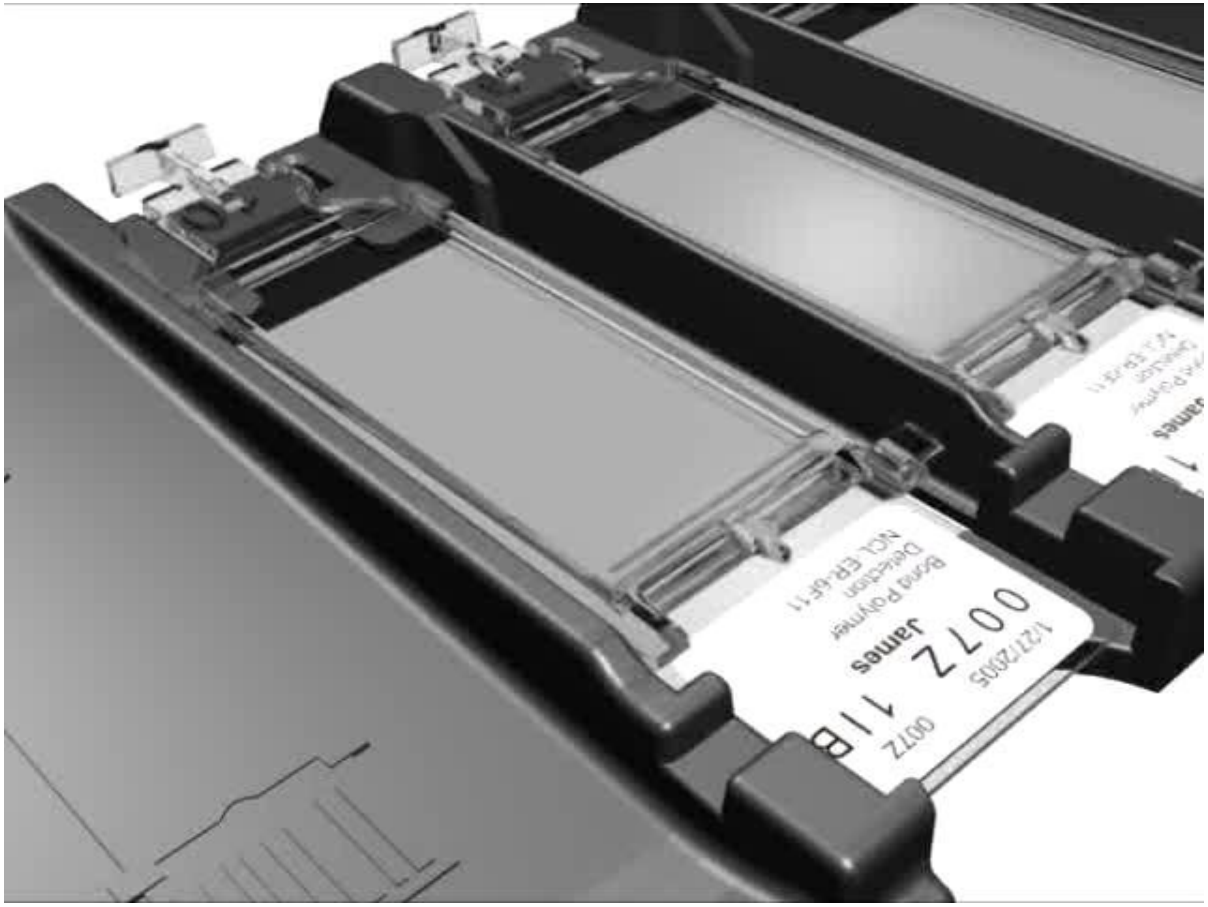
covertile technology

complete and uniform reagent coverage



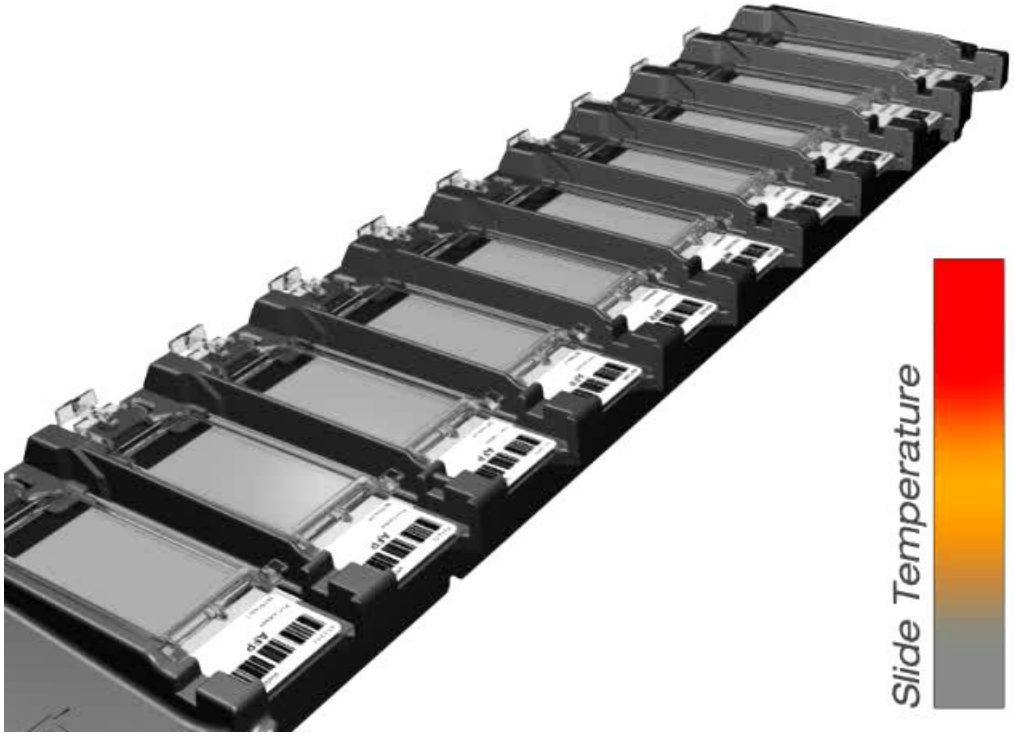
Uniform thickness layer
over entire width of slide

Bond™ Covertile Technology – "Reagent Replacement"



Bond™ Continuous Batch Processing

- Separated heat plate



flexible protocols

create your own staining aesthetic

- Edit, delete or add steps
- Modify pre-treatment and staining steps

Condition enables to be adjusted by the minimal scale for 1 sec and 1℃

protocol editor
- choose an existing protocol or create your own
- replace bond reagent steps
- optimize for best results with difficult antibodies

optical character recognition (OCR)

run slides no matter what

- Human **and** machine readable
- Failsafe
- Even work with hand-written labels

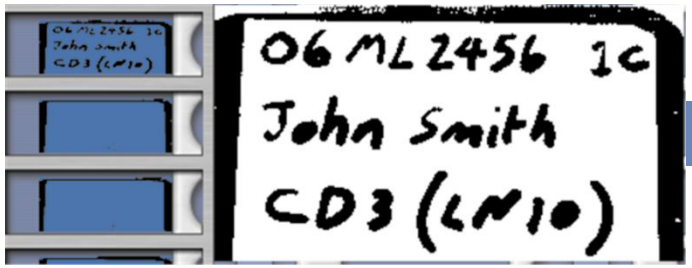


Image of slide label presented to user

flexible slide reader to suit any lab
- digital image capture
- works with any coverslipper
- stored permanently in database
- works with any slide label

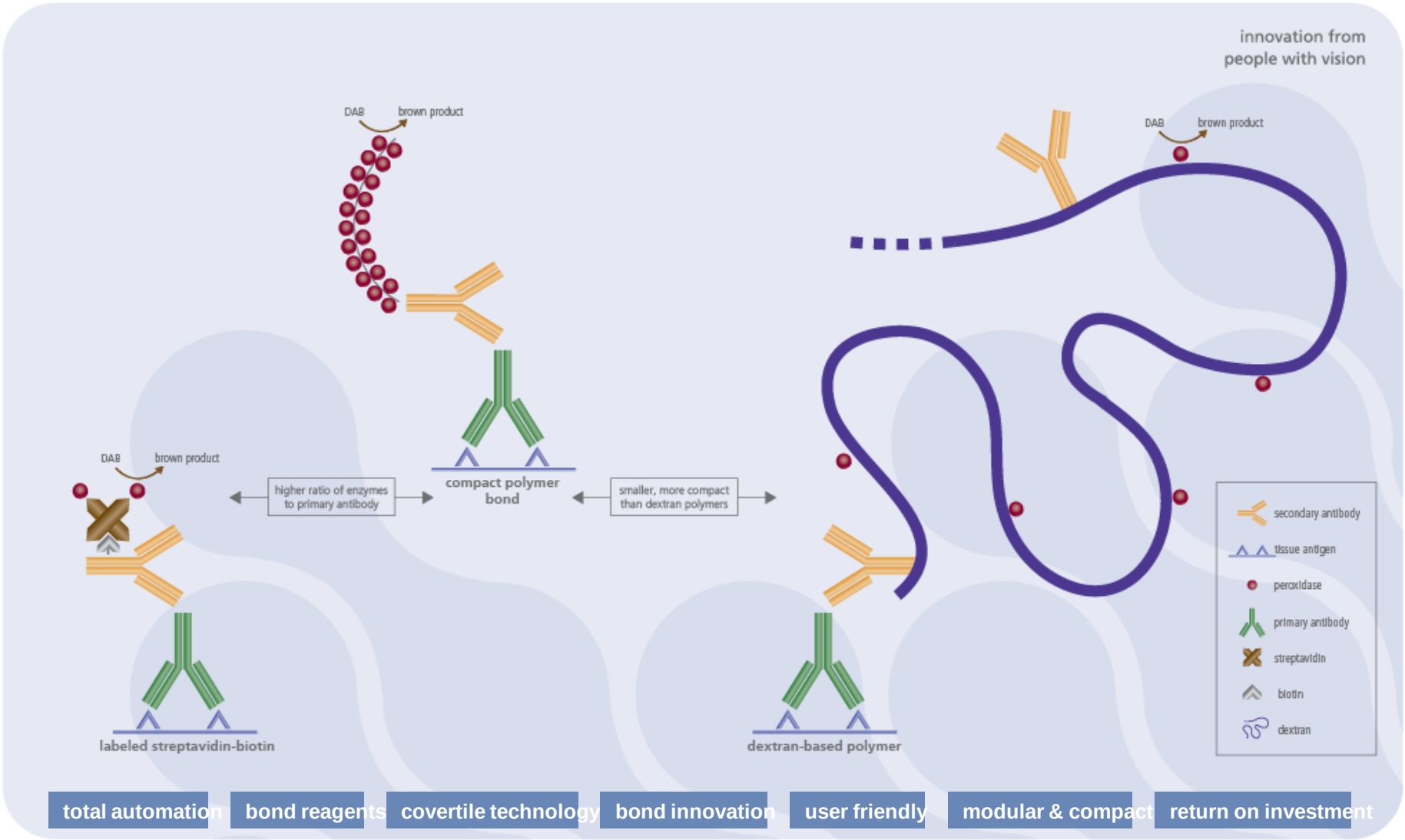
liquid level sensing (LLS)

tracking and monitoring of reagents

- Only starts run if enough reagents are available
- Alerts when reagents are low or waste is full
- Inventory tracking

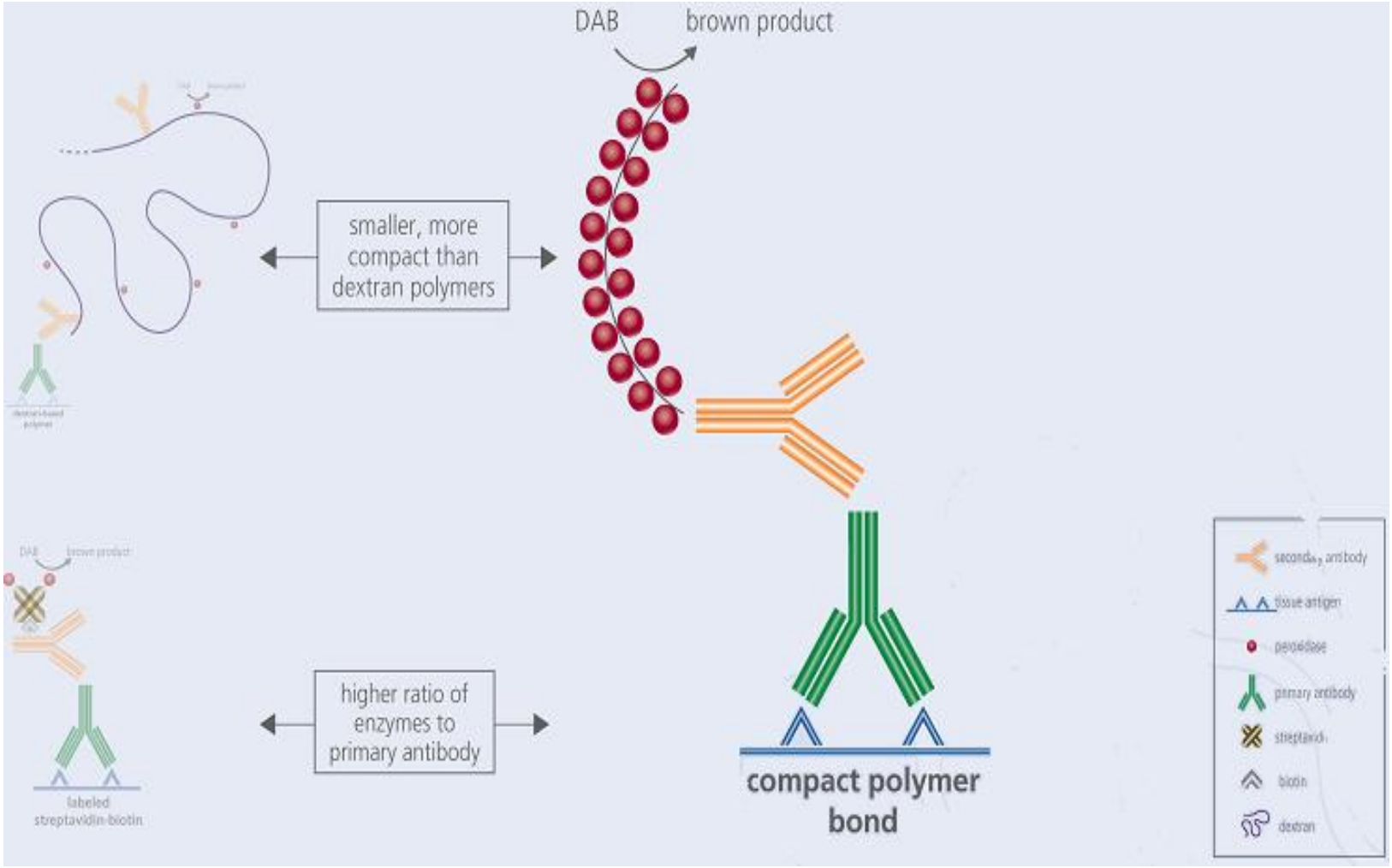
LLS
- robotic arm
- liquid sensing probe tip
- capacitive transducer

bond reagents



total automation | bond reagents | covertile technology | bond innovation | user friendly | modular & compact | return on investment

Compact Polymer Detection Systems



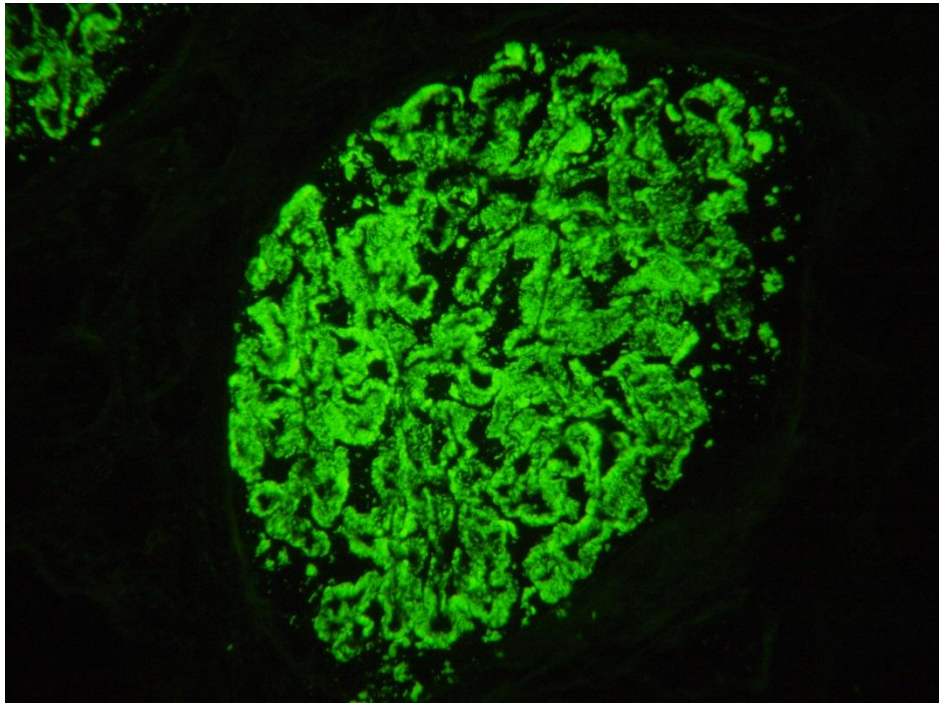
Delay start

- Delay start enables to plan starting at any time.

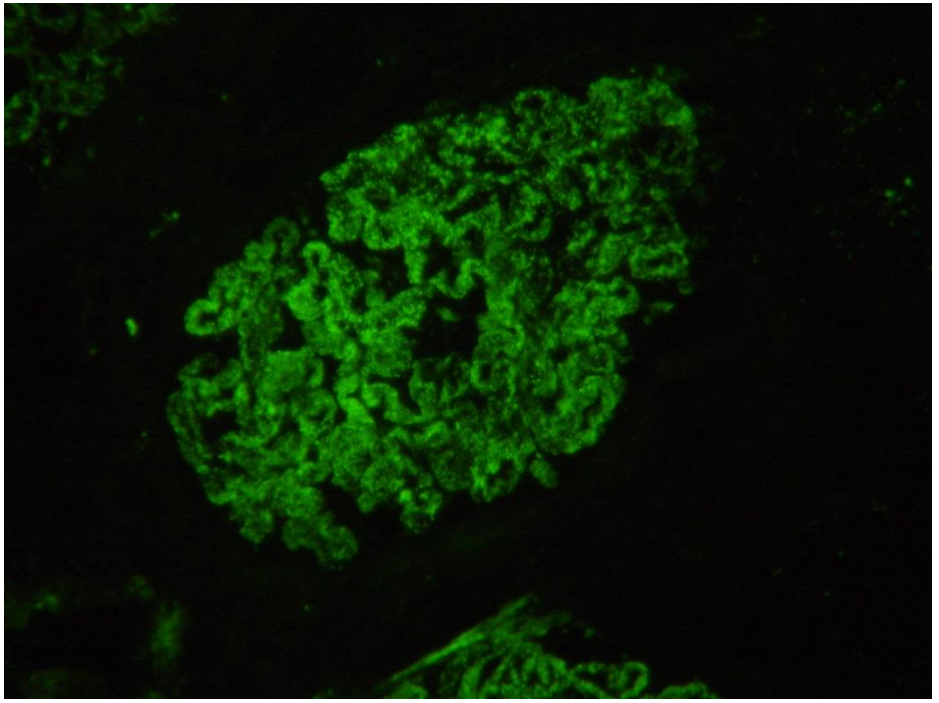
Bond™ Technology Items

- IHC
- IFA
- CISH
- Double stain (IHC/IHC or IHC /ISH)
- FISH

IF

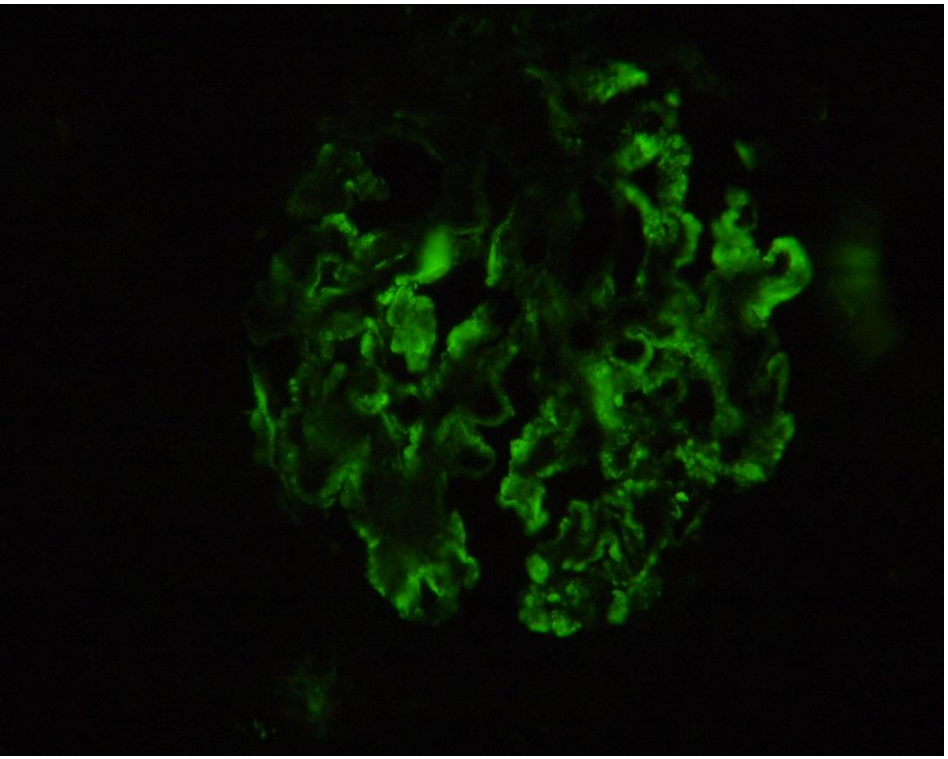


IgG

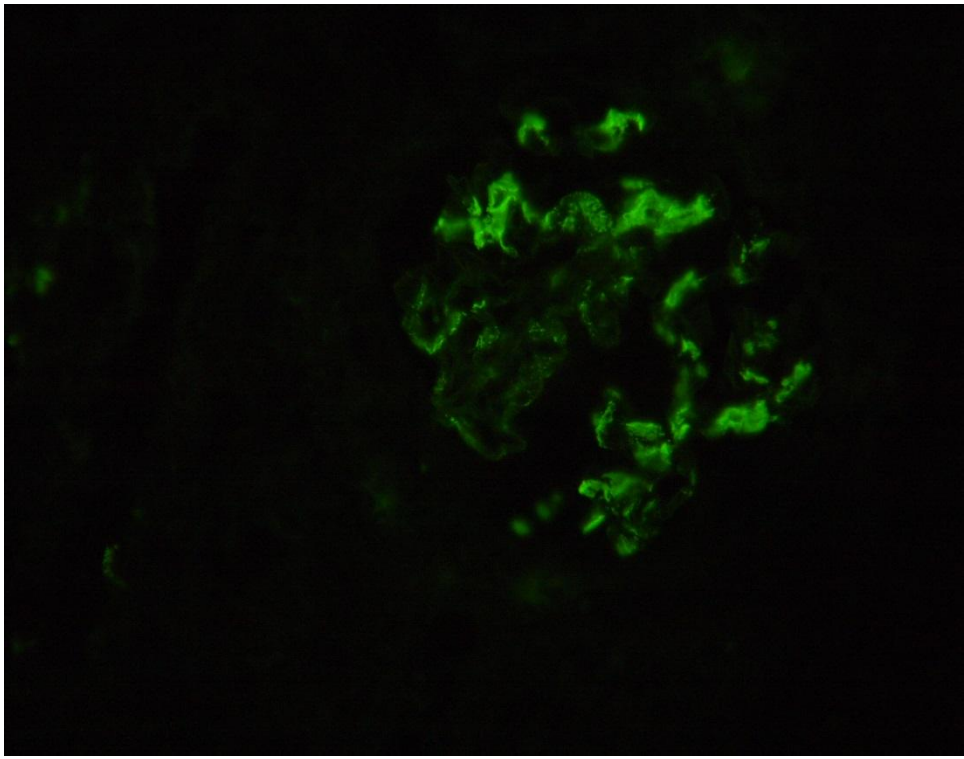


C3

IF

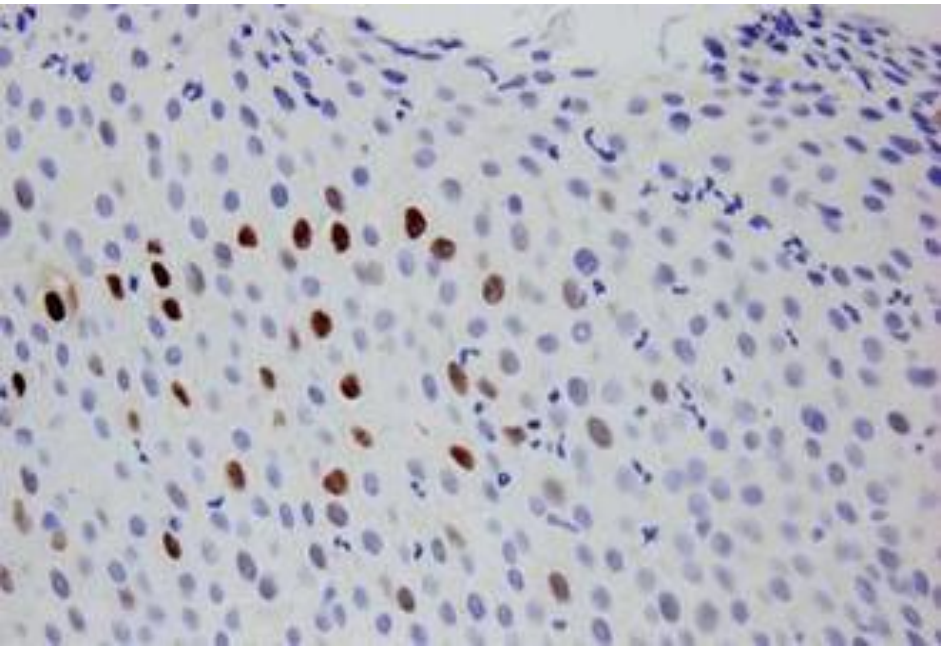


IgM

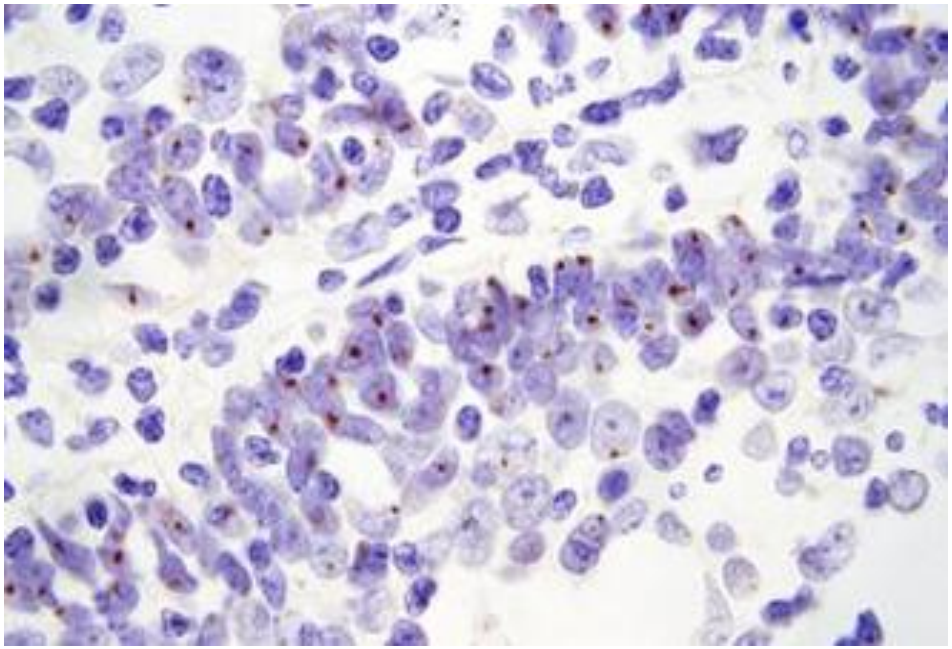


IgA

CISH-HPV

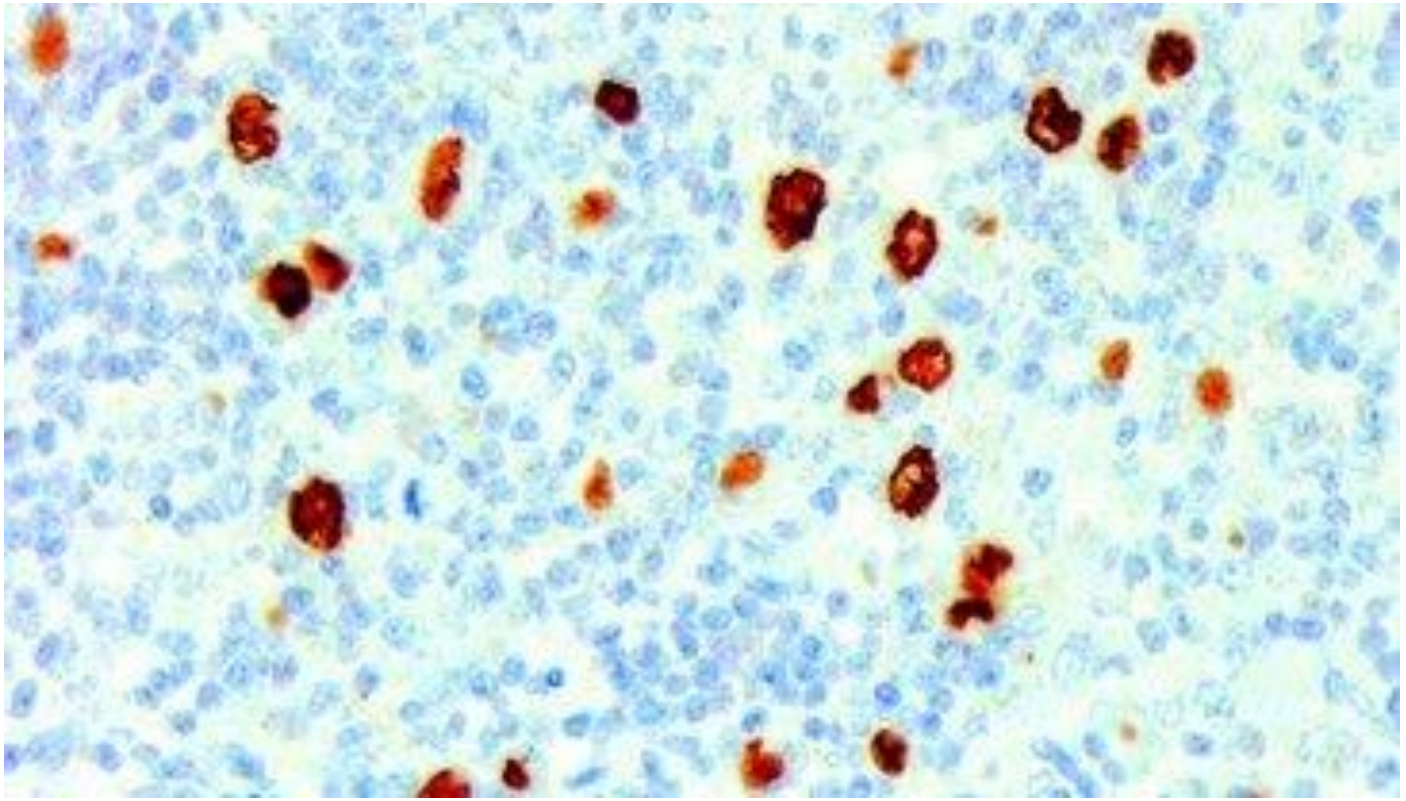


Cervical tissue (CIN1): in situ hybridization for HPV, subtype 6 and 11

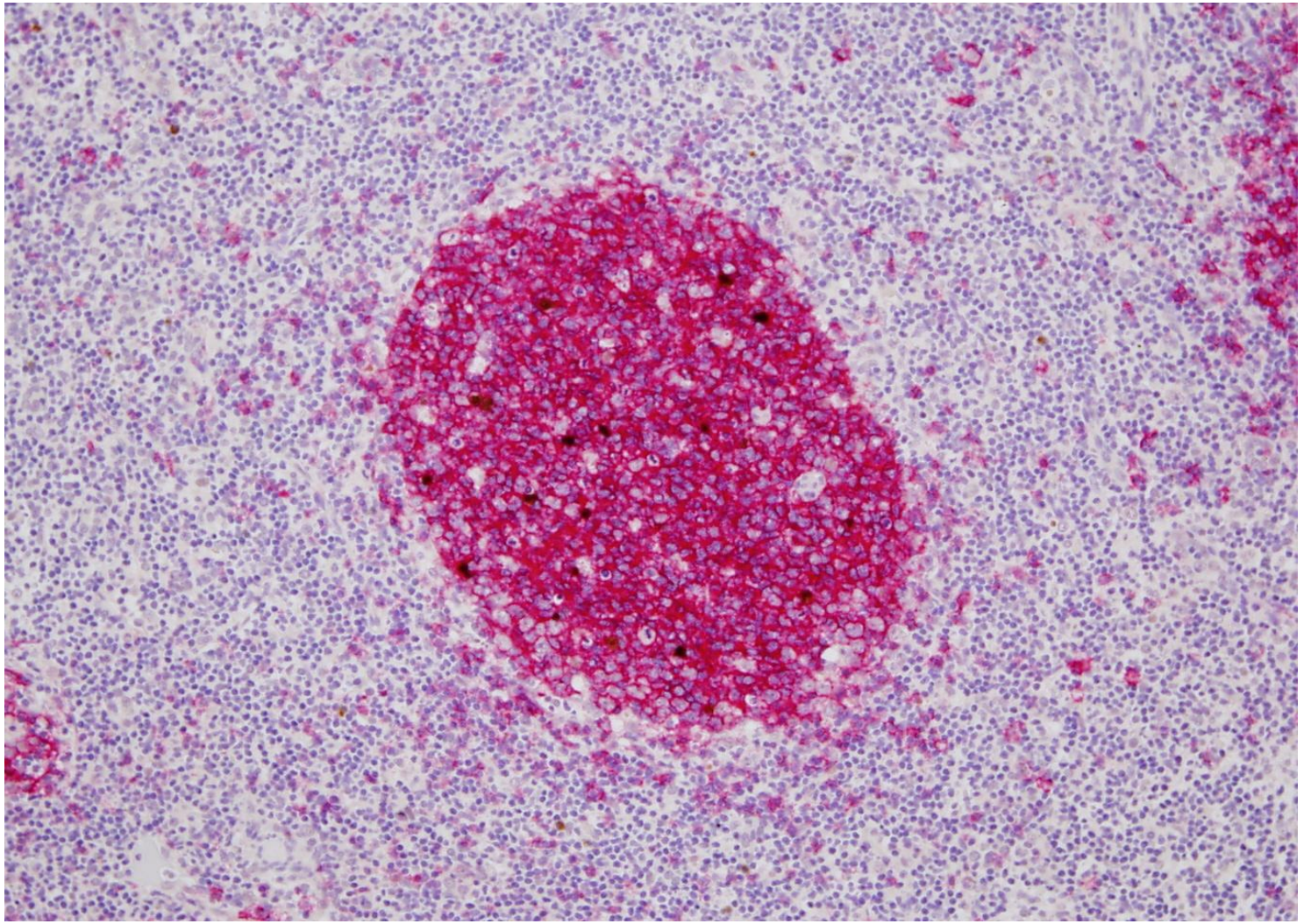


Cervical tissue, abnormal epithelia (CINII) stained with HPV (subtypes 16, 18, 31,33, 51)

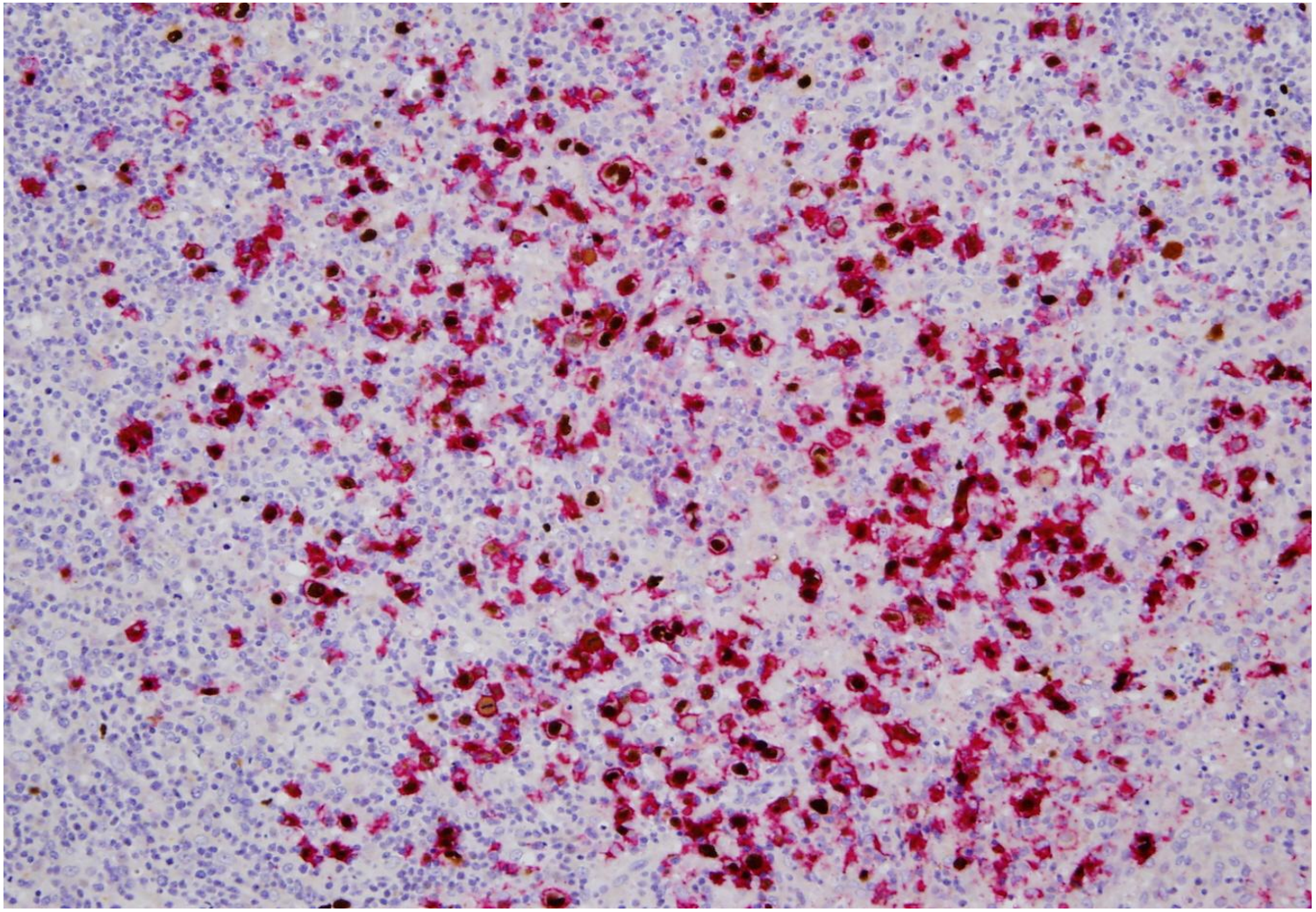
CISH-EBER



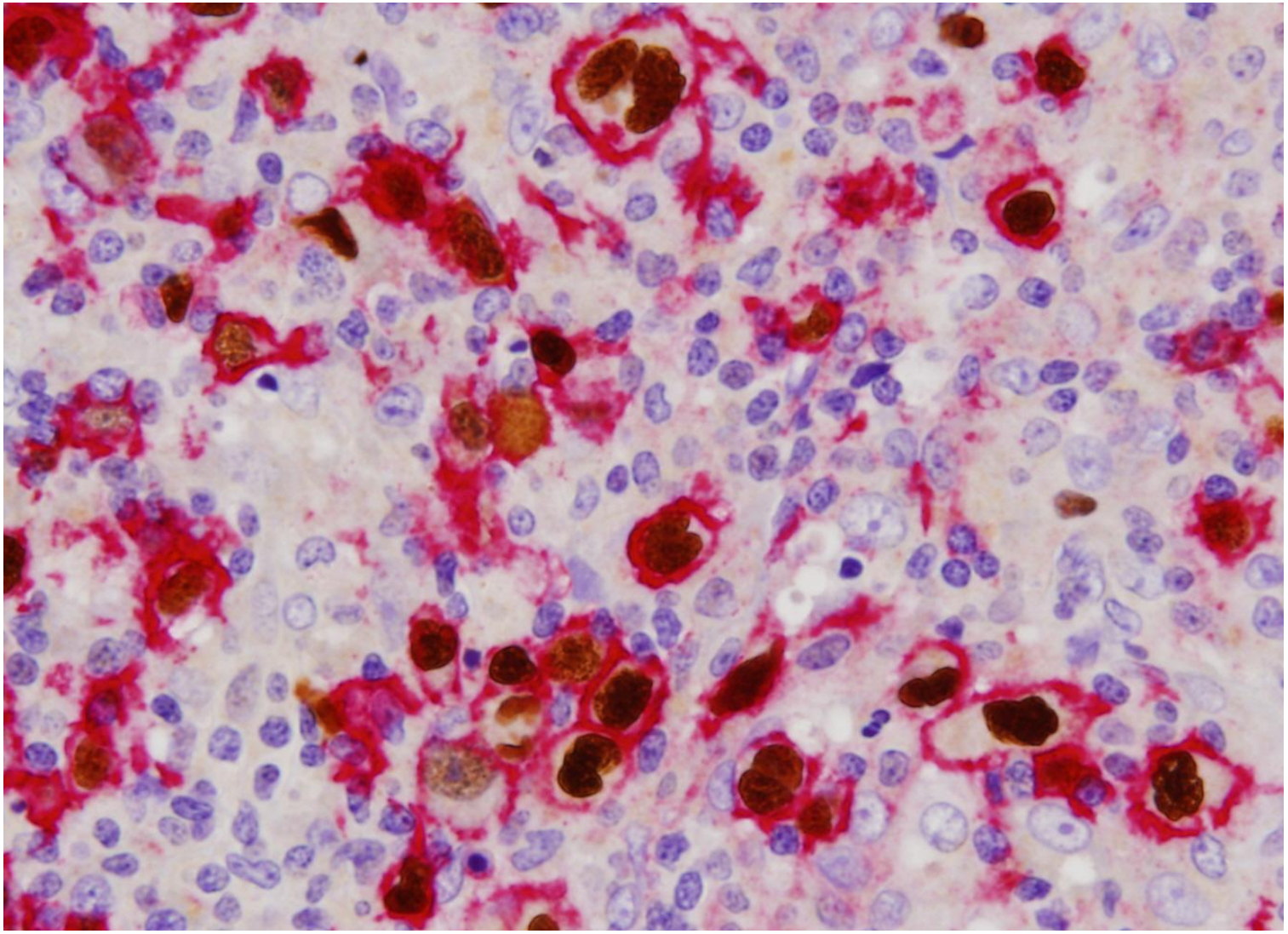
Double stain-EBER/CD20



Double stain-EBER/CD20

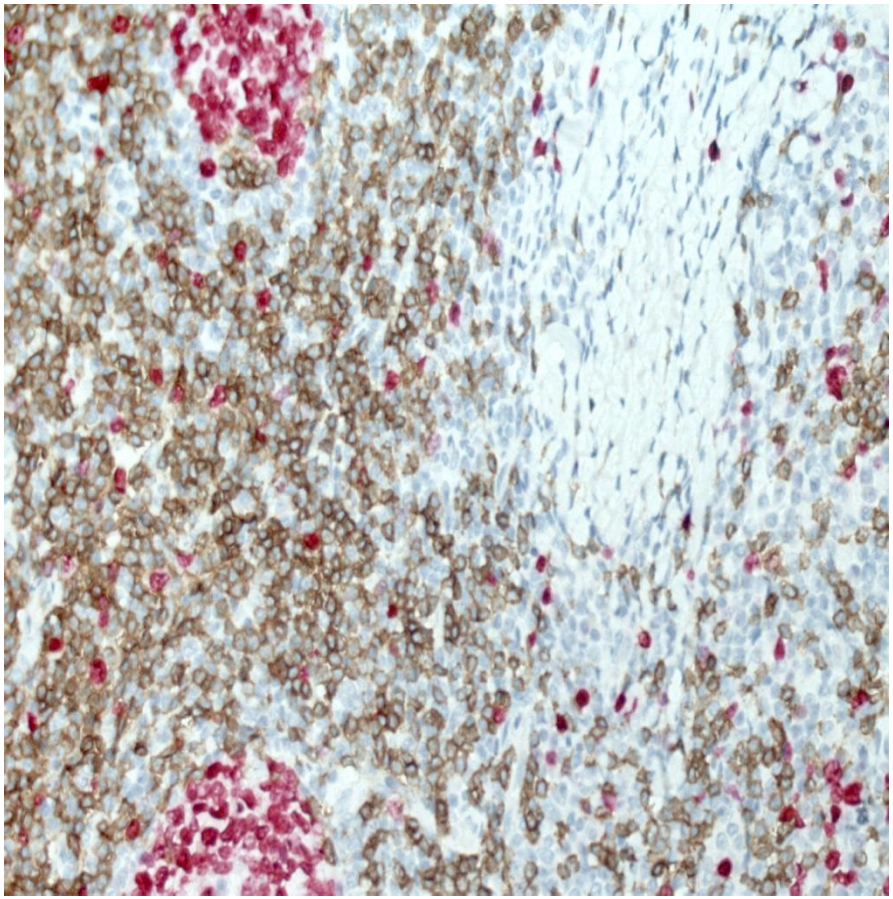


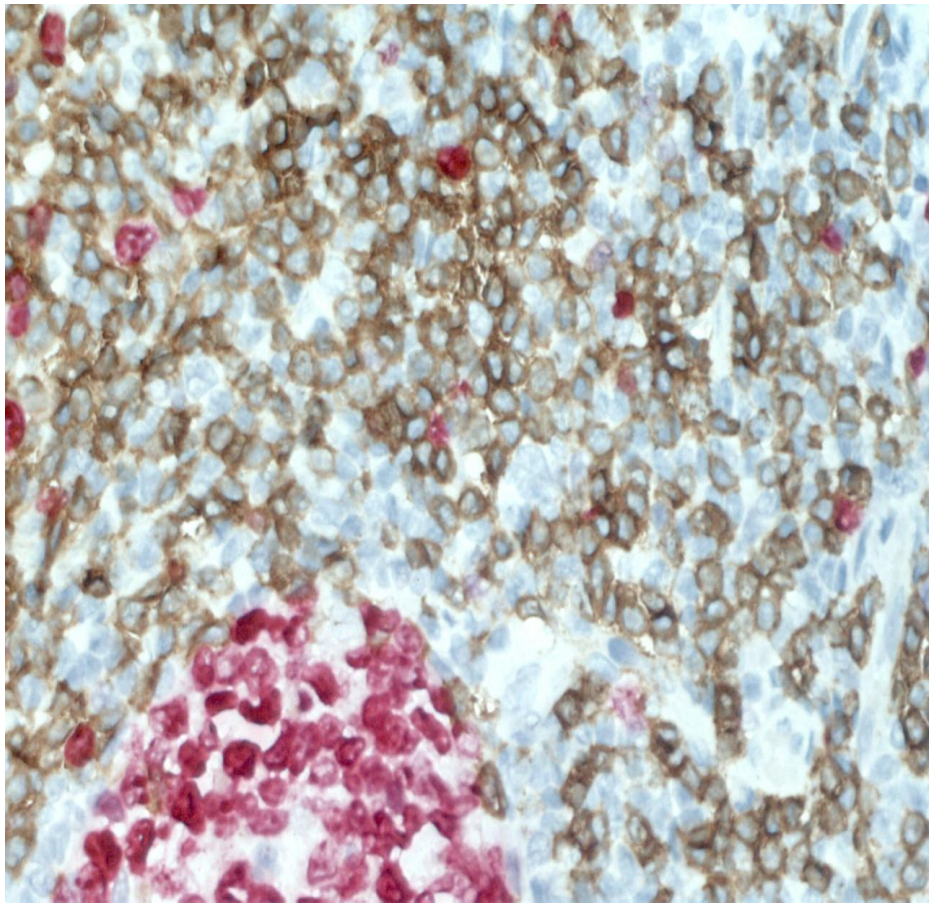
Double stain-EBER/CD20



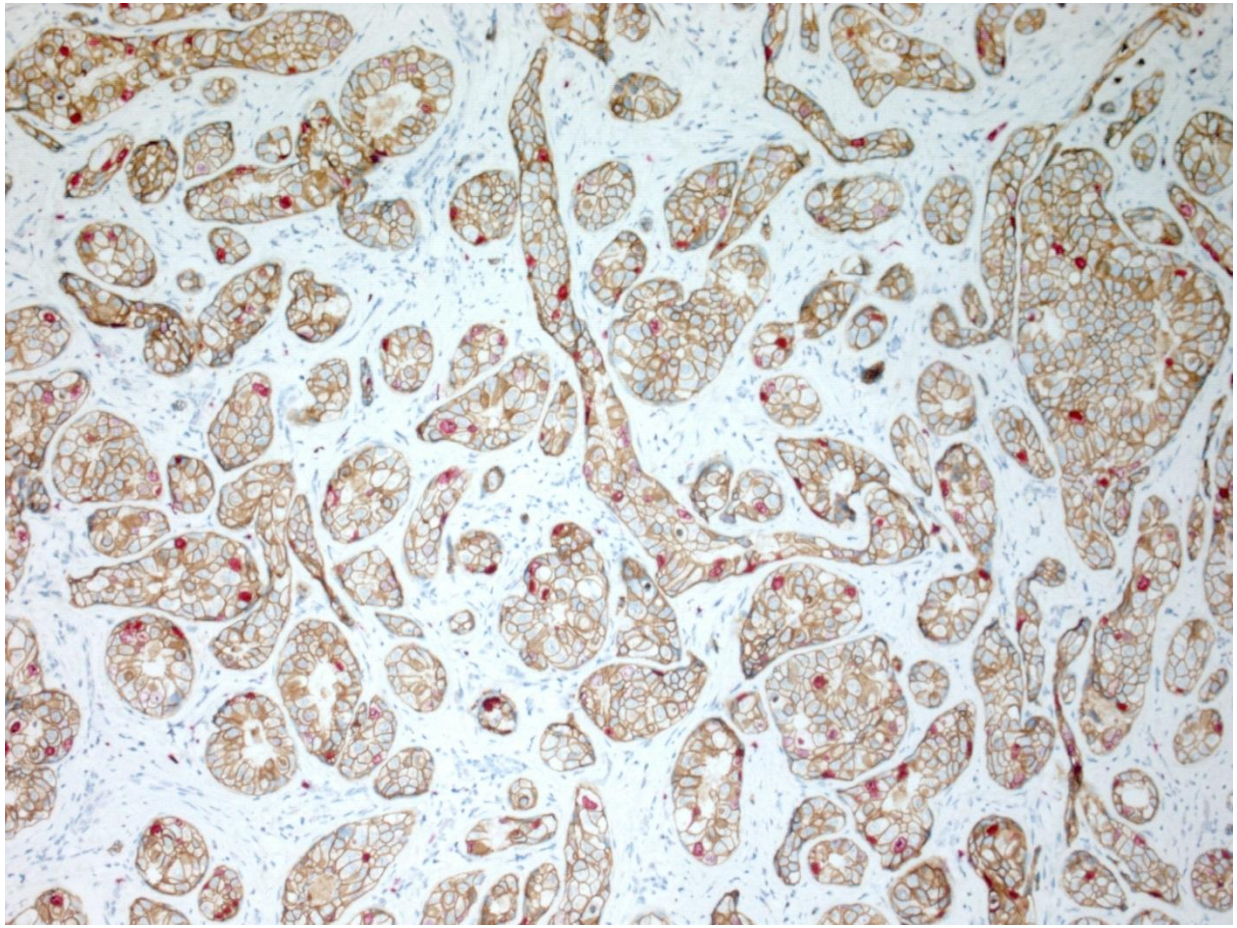
Double stain - Ki-67/CD3

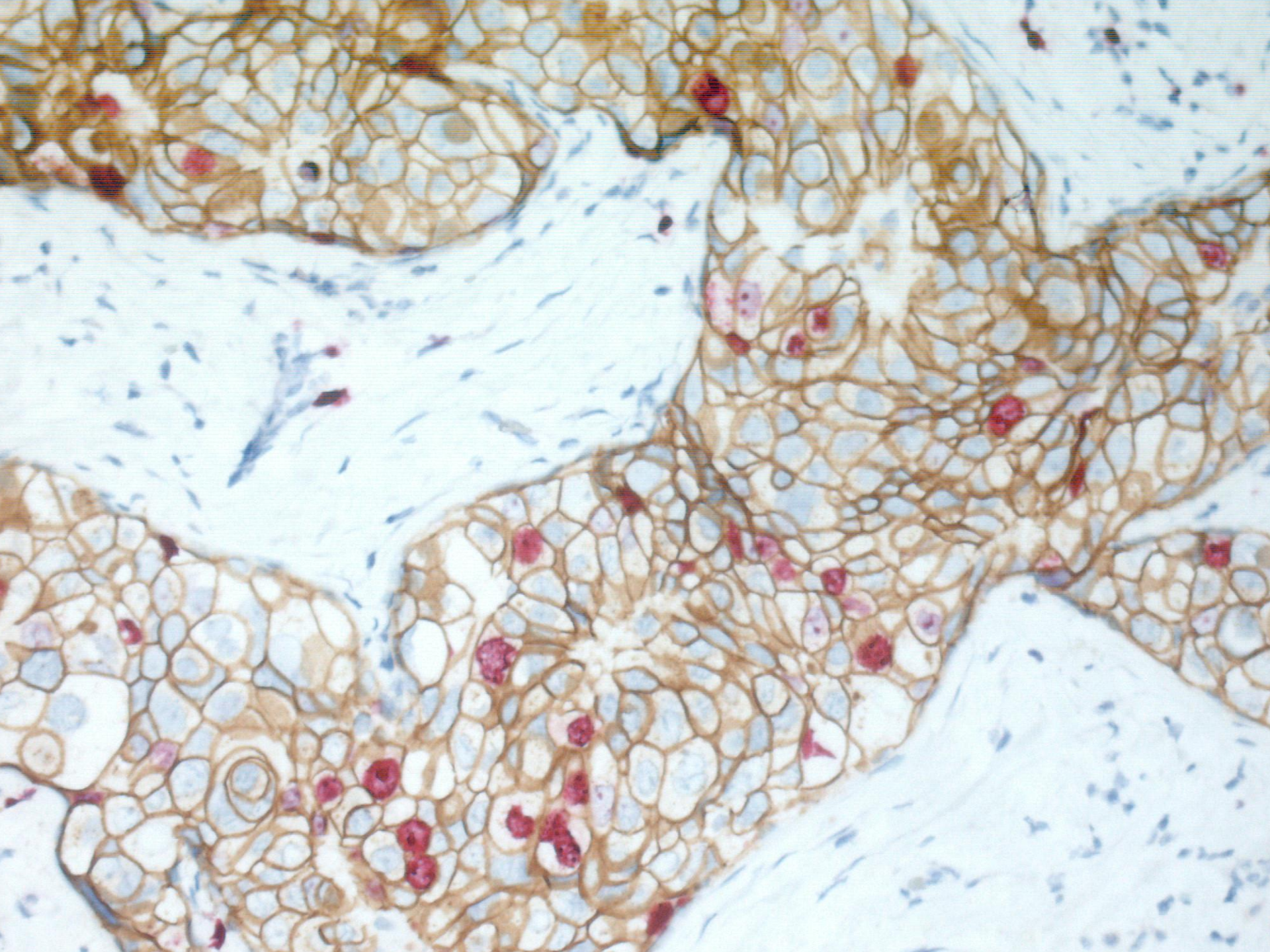
Images of successful stains with KI67 (1:1000) / CD3 (1:300), ER2, 20 min



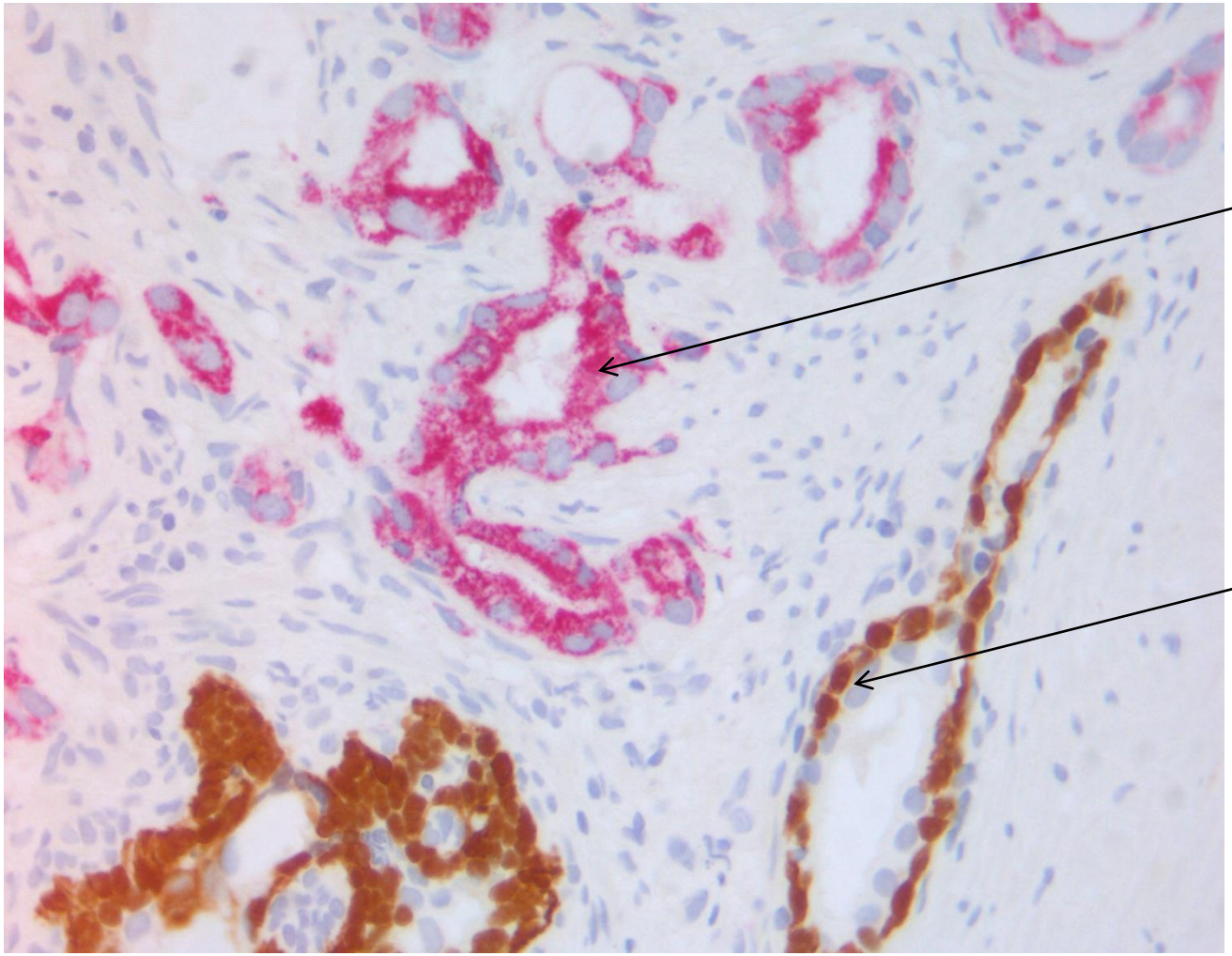


Double stain - E-cadherin/Ki-67





Slide Stained with PIN-4*



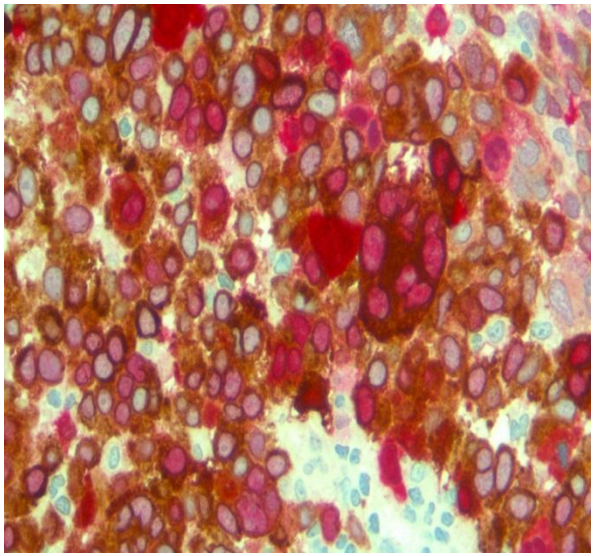
Prostatic carcinoma stained with p504s

Normal glands stained with CK5/CK14 and p63

*Biocare

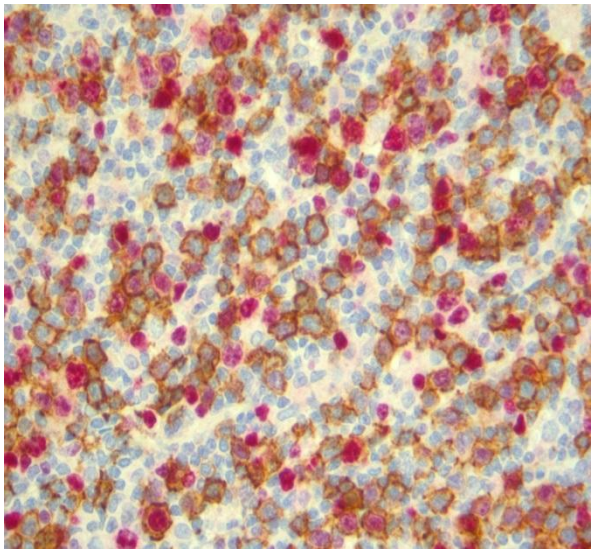
Why use multi-staining techniques?

- More information from each slide
- Assess the topographic relationship of targets
- Information on possible cell-to-cell spatial contacts of different cell types



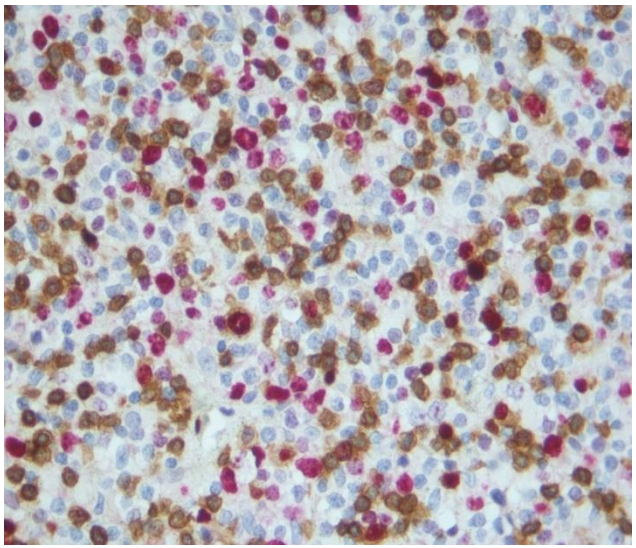
Novocastra Melanoma

Melan A /Tyrosinase / S-100



Novocastra Lymphoma

CD20 / Ki67



Novocastra Lymphoma

CD3 / Ki67

Sequential staining

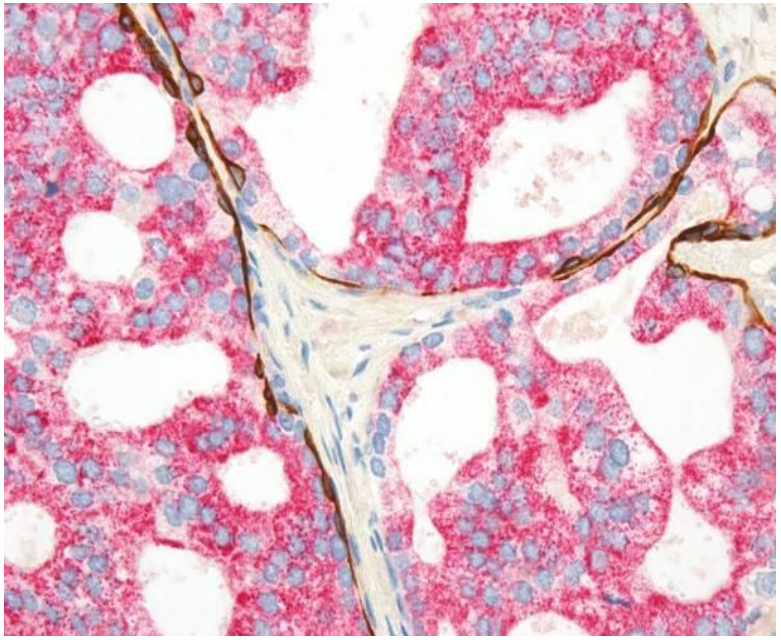
One staining procedure follows another

Advantage:-

- Cross reactivity is avoided

Disadvantages:-

- Long staining protocol
- Risk of incorrect double staining
- Potential for spurious double stained structures
- Risk of denaturing epitopes of antigens to be visualized sequentially
- Risk that the first chromogen (DAB in particular) may shield other targets
- Not all reaction products will survive the rigorous washing required to remove antibodies
- Added complexity



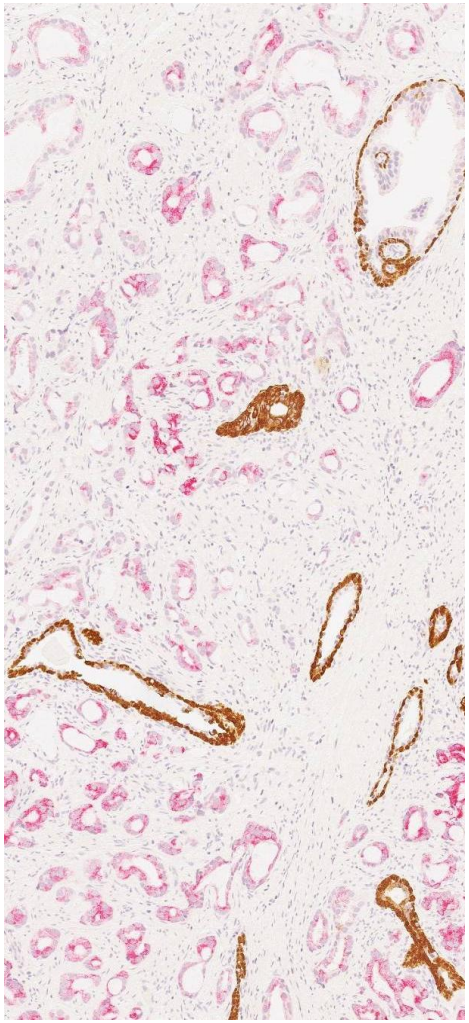
Bond Polymer Double Staining
P504s (Refine Red) and CK5 (Refine)

Fully automated parallel dual staining

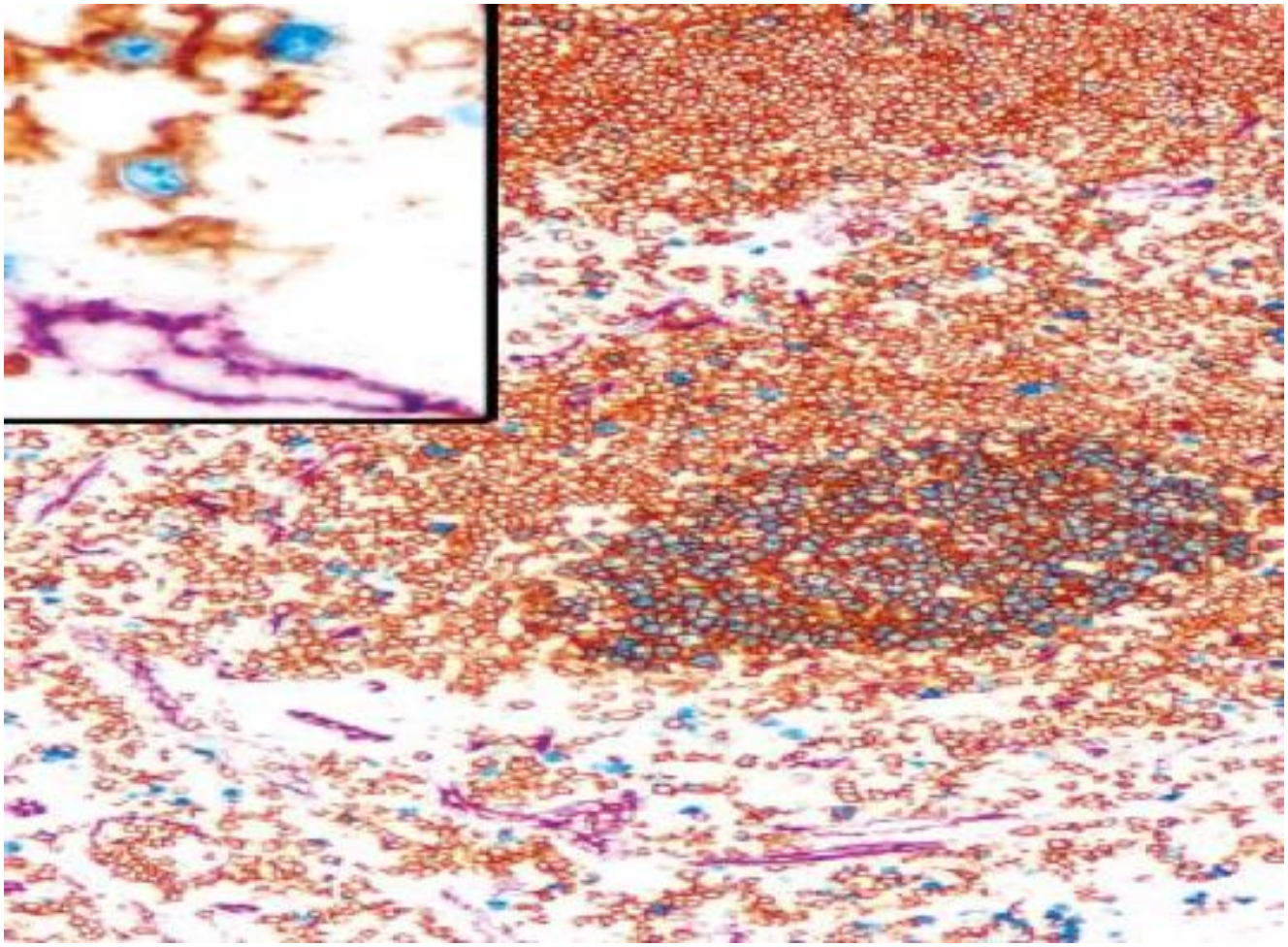
–Leica Microsystems will launch the first fully automated detection system on BOND that will automate dual chromogen staining in a one-step process:-

- Two chromogens in one detection system
- Multiple antibodies on a single slide
- Intense staining with a clean background
- Flexible antibody cocktail options – Novocastra Melanoma & Lymphoma cocktails post launch
- Reduction in TAT and cost

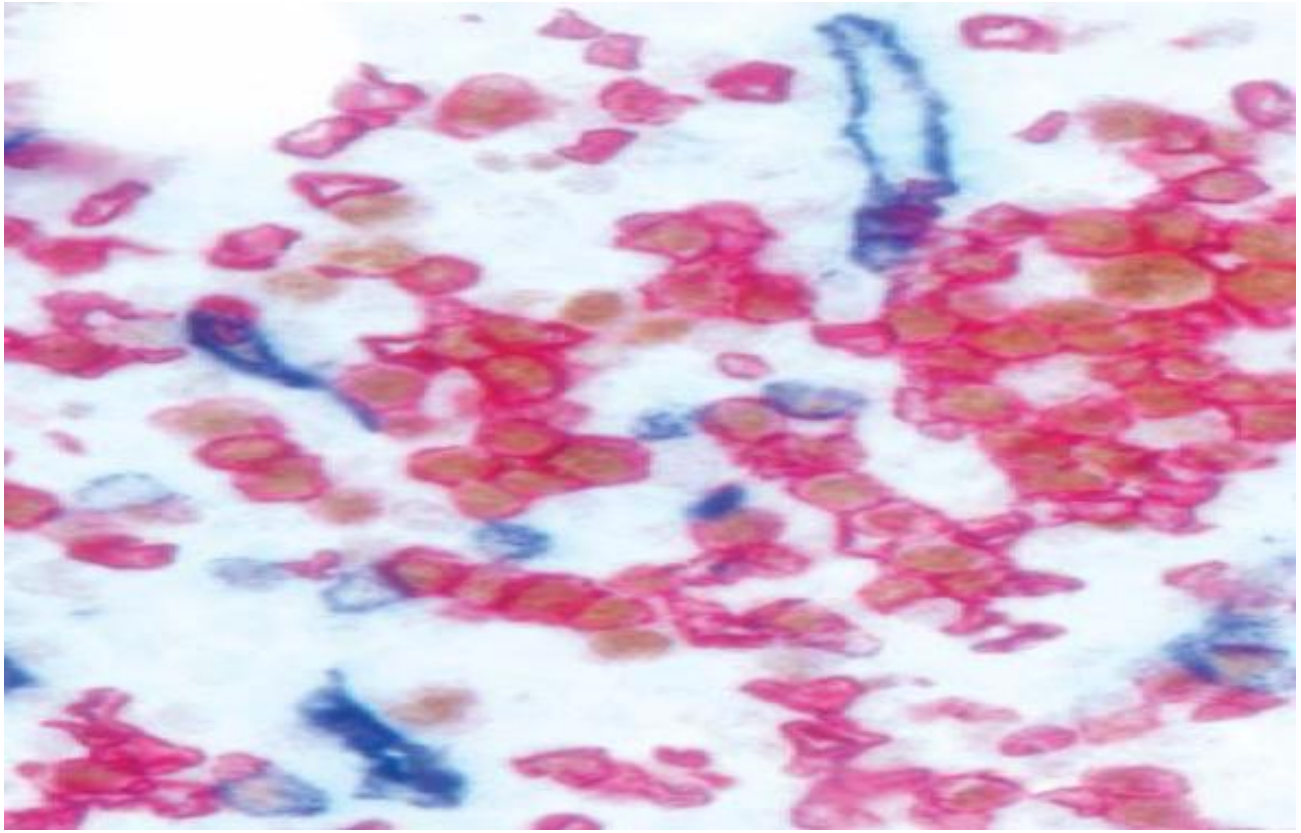
–Multiply your capabilities



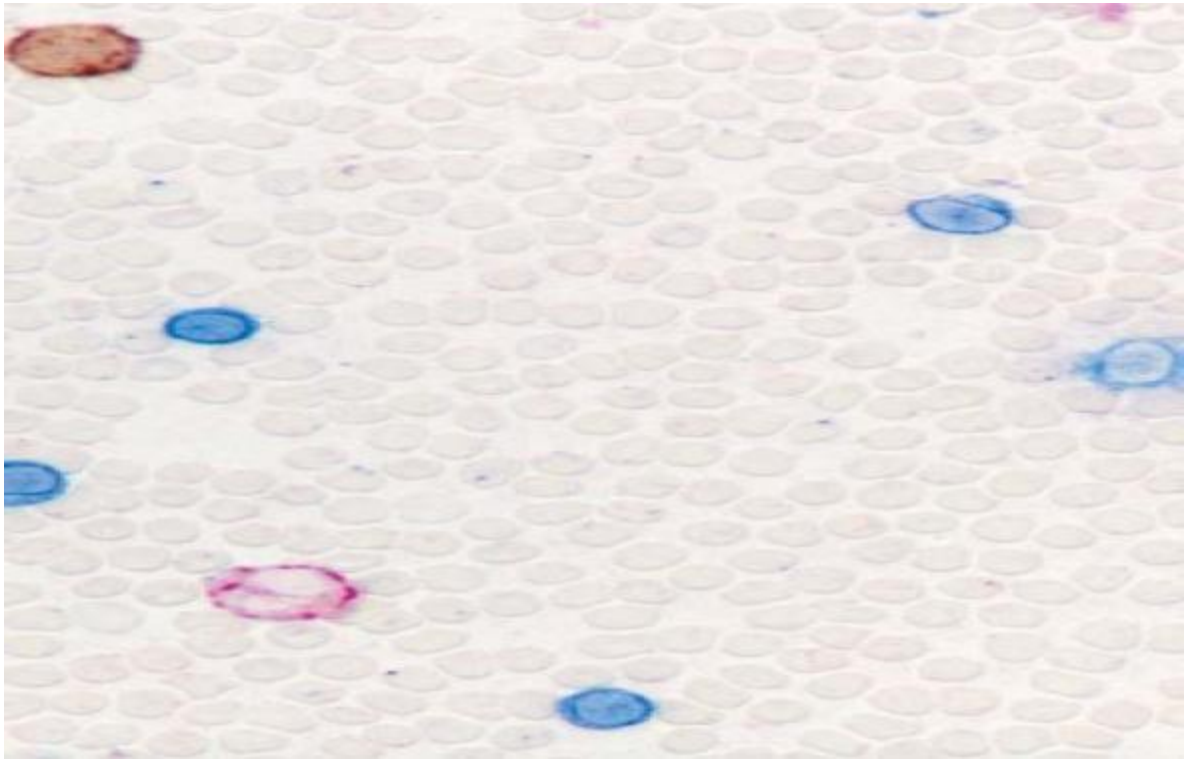
CD20-positive B cells were revealed fi rst by the immunoperoxidase procedure (brown), and immunoalkaline phosphatase staining was then performed for CD34 (purple) and Ki67 (blue).



Automated triple immunostaining of a normal human bone marrow trephine shows nuclear expression of GATA-1 (brown) in some erythroid cells which express Glycophorin A (red). CD34 (blue) labels vascular endothelium and some mononuclear cells.



B cells (CD20, brown), T cells (CD3, blue), and monocytes (CD68, red)

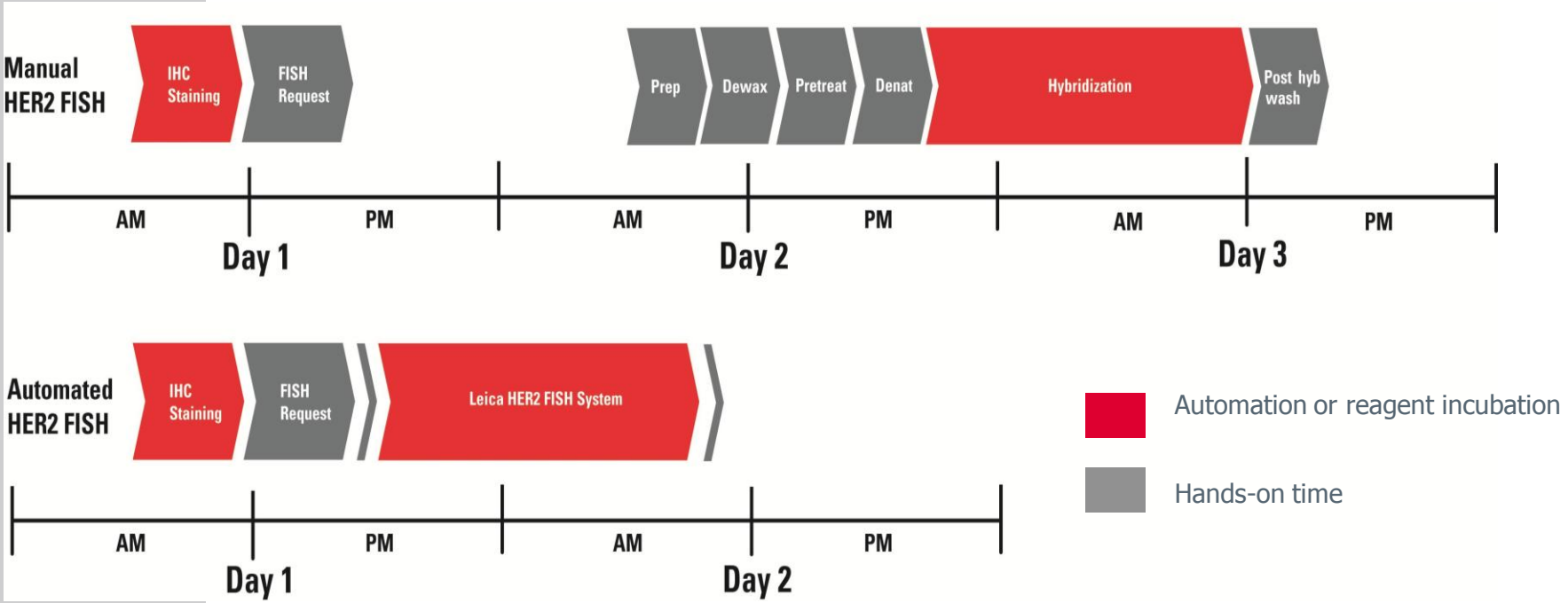


Leica HER2 FISH System for BOND™

This product uses PathVysion FISH probes supplied by Abbott Molecular Inc.

Leica HER2 FISH System 95.10230 rev b 05/2011. This product is not for sale in the USA.

Typical HER2 Workflow



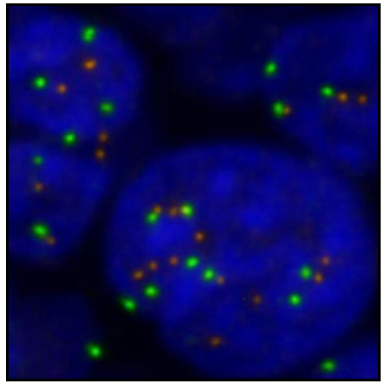
- The Leica HER2 FISH System can deliver next day HER2 results, up to 24 hours faster than current HER2 workflow
- Patient cases can be continuously processed on the Leica BOND systems without the delay required to batch cases for manual HER2 FISH staining

Patient to Pathologist

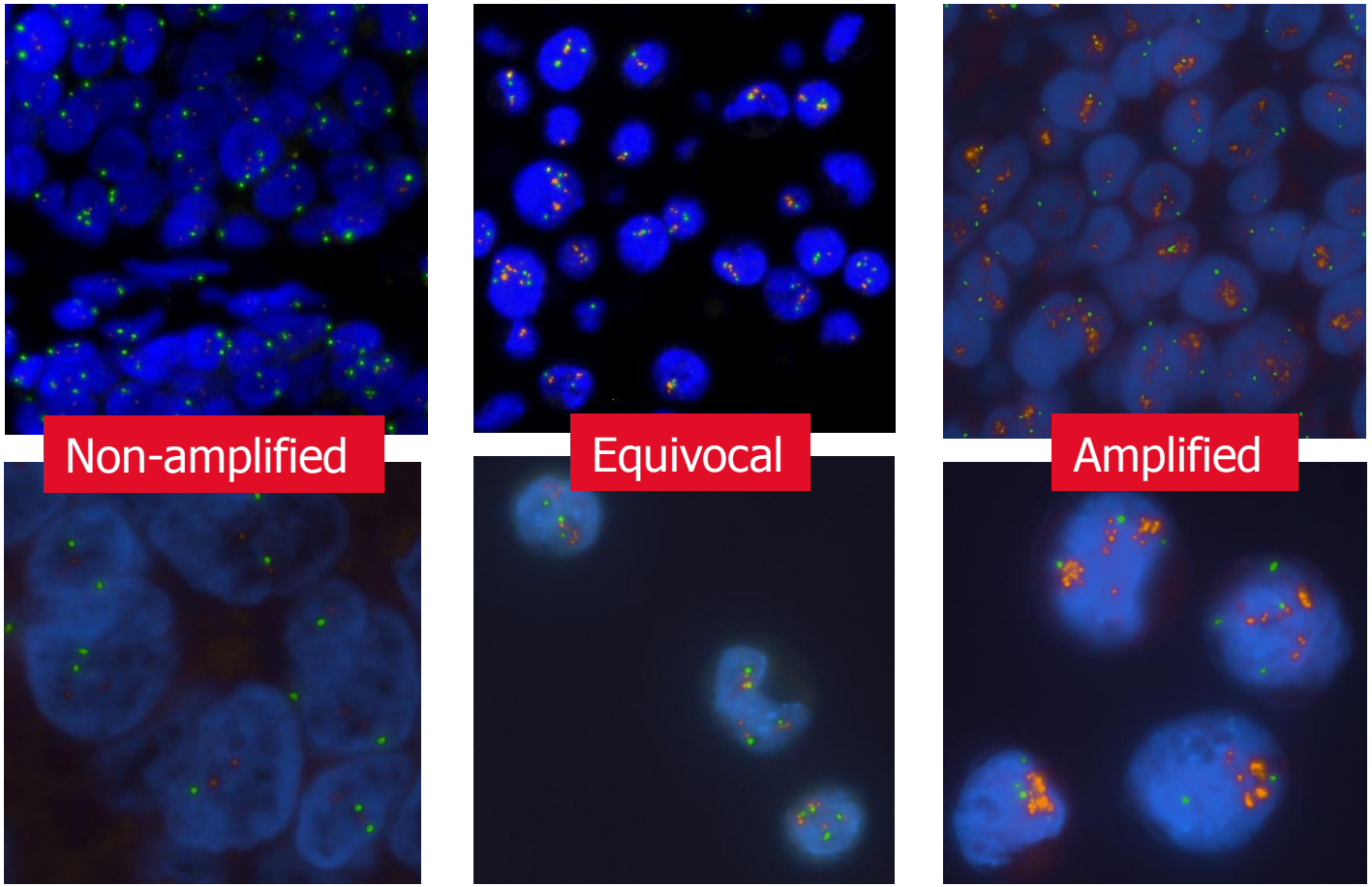
EASY

- Eliminate complexity and increase standardization through automation
- The Leica HER2 FISH System provides:
  - Full automation of HER2 FISH using PathVysion probes obtained from Abbott Molecular
  - PathVysion HER2/CEP17 probes are incorporated into the Leica HER2 FISH kit
  - Familiar operation on the easy-to-use Leica BOND System

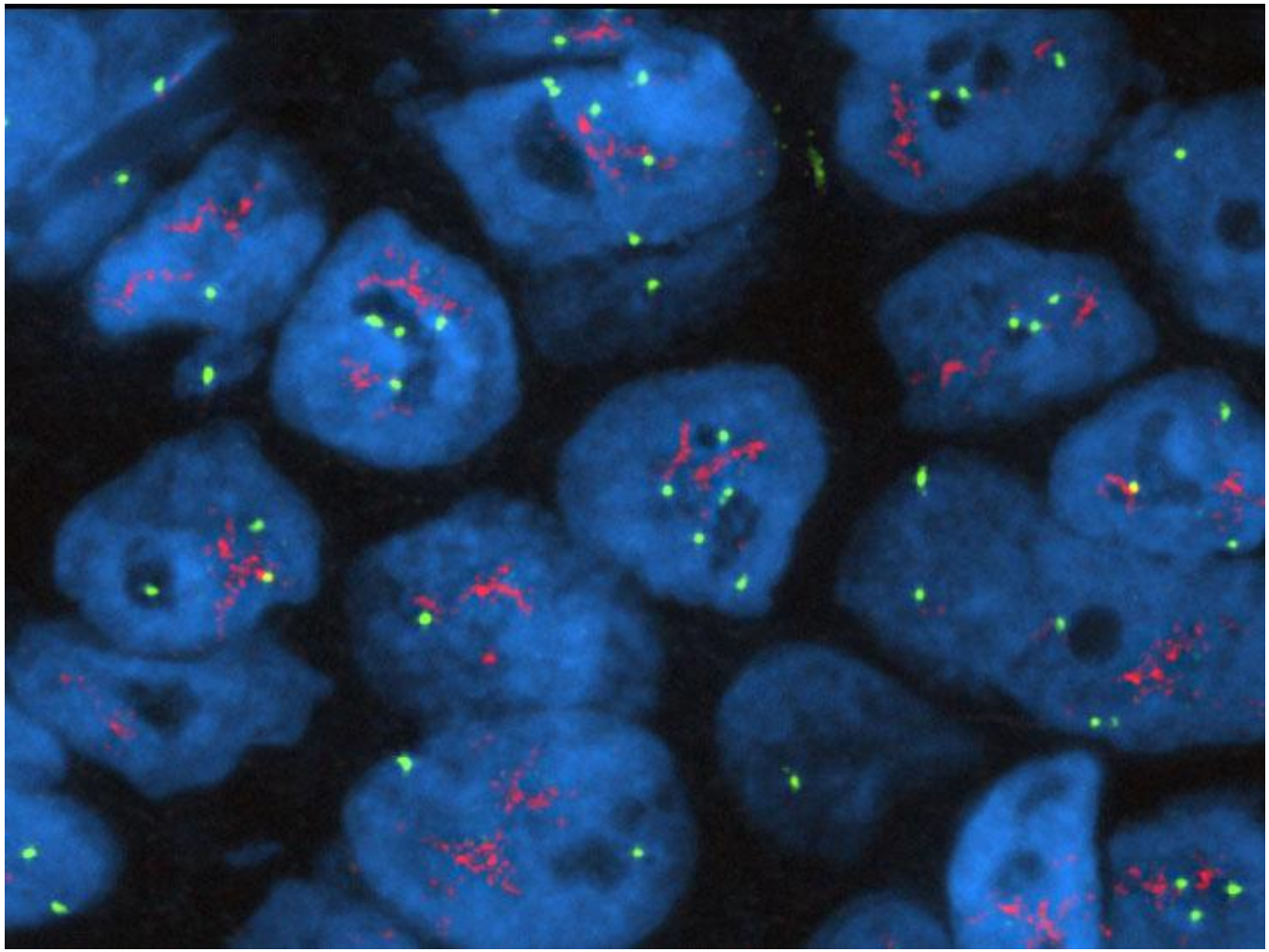
With the Leica HER2 FISH System, laboratory staff will find it easy to produce the consistent, high-quality stained slides that pathologists rely on



Leica HER2 FISH System Images



FISH Positive



Concordance Data for Leica BOND Max

- In an independent study comparing the results of the Leica HER2 FISH System to the Abbott Molecular PathVysion® HER-2 DNA Probe Kit on 300 clinical breast cancer specimens, the Leica HER2 FISH System was found to be highly concordant to the current gold standard, FDA approved HER2 FISH test

		Abbott Molecular PathVysion HER-2 DNA Probe Kit		
		Positive ≥2.0	Negative <2.0	Total
Leica HER2 FISH System Leica BOND Max	Positive ≥2.0	102	1	103
	Negative <2.0	1	196	197
	Total	103	197	300

- Overall Concordance (95% CI) = 99.33% (97.61 - 99.92%)
- The cohort consisted of 75 3+, 150 2+ and 75 0/1+ previously characterized HER2 IHC cases

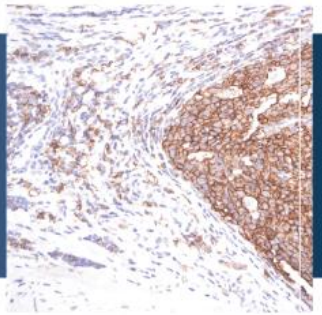
龐德生技Antibody Bank

1.	α-AT	45.	CD38	89.	Galectin3	133.	MSH2
2.	ACTH	46.	CD43	90.	Glucagon	134.	MSH6
3.	Actin(HHF35)	47.	CD44	91.	GFAP	135.	MUM-1
4.	Actin(SM)	48.	CD45RO	92.	GCDFP15	136.	Myo-D1
5.	AFP	49.	CD56	93.	GranzymeB	137.	Myeloperoxidase
6.	ALK	50.	CD57	94.	GH	138.	Myogenin
7.	AMACR (P504s)	51.	CD61	95.	Glutamin synthetas	139.	Myosin heavy chain
8.	Androgen receptor (AR)	52.	CD68	96.	Glypican-3 (GPC-3)	140.	Neurofilment
9.	Arginase-1	53.	CD79a	97.	H-Caldesmon	141.	NSE
10.	B-Amyloid	54.	CD99	98.	HBcAg	142.	P16
11.	Bcl-2	55.	CD117	99.	HBsAg	143.	P27
12.	Bcl-6	56.	CD123	100.	HBME-1	144.	P40
13.	Beta-catenin (B-CAT)	57.	CD138	101.	Her-2	145.	P53
14.	Beta-HCG	58.	CD163	102.	HHV8	146.	P57
15.	Ber-EP4	59.	CDK4	103.	HPV	147.	P63
16.	BF-1	60.	CDX2	104.	hPL	148.	PD-1
17.	BK virus	61.	CEA	105.	HGAL	149.	PHH3
18.	CA125	62.	Chromogranin A	106.	HPV 16/18	150.	PLAP
19.	CA199	63.	CK AE1/AE3	107.	HMB45	151.	PMS2
20.	Calcitonin	64.	CK5	108.	Hepa-1(HSA)	152.	PSA
21.	Calretinin	65.	CK5/6	109.	Heat shock protein 70	153.	PR
22.	Carbonic Anhydrase IX	66.	CK7	110.	Helicobacter pylori	154.	PTH
23.	Calponin	67.	CK8	111.	HSV	155.	Pax-5
24.	C4d	68.	CK13	112.	IgD	156.	Pax-8
25.	CD1a	69.	CK14	113.	IgG	157.	Placenta S100 (S100P)
26.	CD2	70.	CK17	114.	IgG4	158.	Prolactin
27.	CD3	71.	CK18	115.	Insulin	159.	PsAP
28.	CD4	72.	CK19	116.	Inhibin	160.	RCC
29.	CD5	73.	CK20	117.	INI-1	161.	Synaptophysin
30.	CD7	74.	CK-HMW (34BE12)	118.	IDH-1	162.	S100
31.	CD8	75.	CMV	119.	IgD	163.	SOX-2
32.	CD10	76.	Collagen4	120.	Inhibin A	164.	SOX-11
33.	CD11b	77.	Cyclin D1	121.	Kappa	165.	SMA
34.	CD15	78.	D2-40	122.	Ki-67	166.	TCR-gamma
35.	CD19	79.	Desmin	123.	Lambda	167.	TdT
36.	CD20	80.	DOG1	124.	LCA	168.	Thrombomodulin
37.	CD21	81.	E-Cadherin	125.	LH	169.	Thyroglobulin
38.	CD23	82.	EGFR	126.	Lysozyme	170.	TLE1
39.	CD25	83.	EMA	127.	MDM2	171.	TFE3
40.	CD30	84.	ER	128.	Melan-A	172.	Treponema
41.	CD31	85.	Factor VIII	129.	Mammaglobin	173.	TSH
42.	CD33	86.	Factor XIIIa	130.	MGMT	174.	TTF-1
43.	CD34	87.	Fli-1	131.	MLH1	175.	Vimentin
44.	CD35	88.	Gastrin	132.	MOC-31	176.	WT-1

Bond-Max 使用單位一覽表

- 台北榮民總醫院 5
- 國立台灣大學醫學院附設醫院 3
- 奇美醫療財團法人永康奇美醫院2
- 長庚醫療財團法人林口長庚醫院 3
- 中國醫學大學附設醫院 4
- 高雄榮民總醫院2
- 高雄醫學大學附設中和紀念醫院 2
- 財團法人佛教慈濟綜合醫院大林分院 1
- 國立台灣大學醫學院附設醫院雲林分院1
- 光田醫療社團法人光田綜合醫院 1
- 國立成功大學附設醫院 1
- 國泰綜合醫院 1
- 長庚醫療財團法人嘉義長庚醫院 1
- 童綜合醫療社團法人童綜合醫院 1
- 財團法人天主教聖馬爾定醫院 1
- 振興醫療財團法人振興醫院 1
- 長庚醫療財團法人高雄長庚醫院 1
- 長庚醫療財團法人基隆長庚醫院 1
- 行政院衛生署豐原醫院 1
- 仁愛綜合醫院1
- 嘉義聖馬爾定醫院1
- 澄清醫院中港分院1





Thank you for Attention today