AUGUST 2025 ISSUE #2

THE CUTTING EDGE

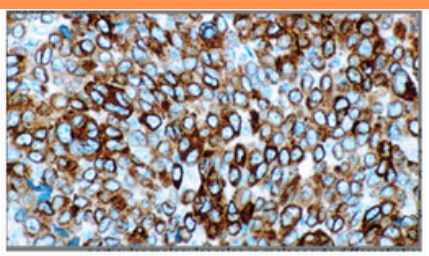
THE OFFICIAL NEWSLETTER FOR
THE TEXAS SOCIETY FOR HISTOTECHNOLOGY

Sun Exposure, Melanoma, and the Expanding Role of IHC

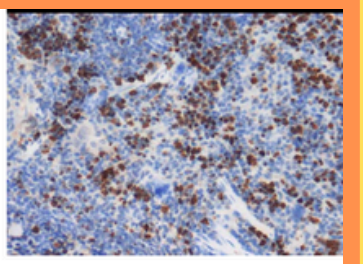
By Toni Lona, MSc., HTL(ASCP)
TSH Newsletter Chair

While summertime brings long days and outdoor fun, it also brings an increase in UV exposure, one of the most significant environmental risk factors for melanoma (a highly aggressive malignancy of melanocytes.) As histotechs, we're key players in the identification, diagnosis, and even treatment monitoring of this deadly disease thanks to the evolving toolbox of immunohistochemical (IHC) markers we apply in the lab.

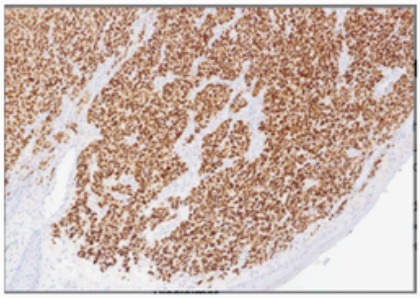
Staining of S-100B from Leica Biosystems. Nuclear and cytoplasmic staining in tumor cells. Clone: EP32



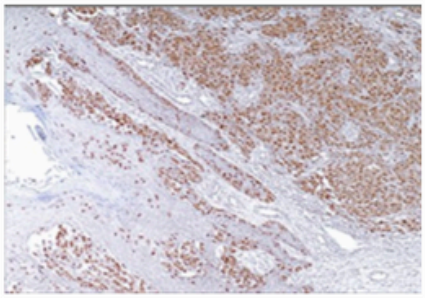
Staining for Melan A from Leica Biosystems. Note the melanoma showing cytoplasmic staining of tumor cells. Clone: A103



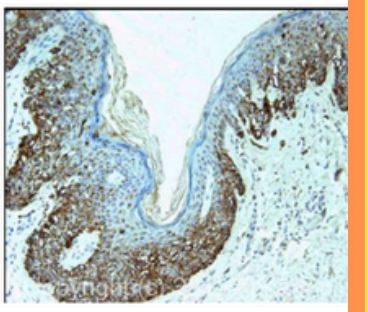
Staining for Ki-67 from Abcam



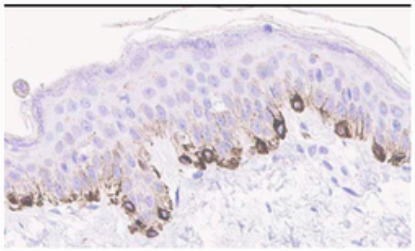
Staining of SOX10 from Leica Biosystems. Clone SOX10/991.



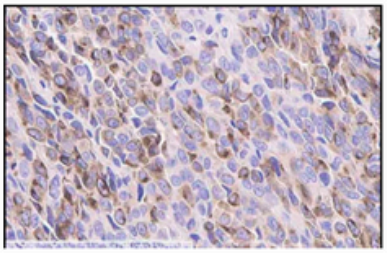
Staining for Microphthalmia Transcription Factor (MiTF) from Leica Biosystems



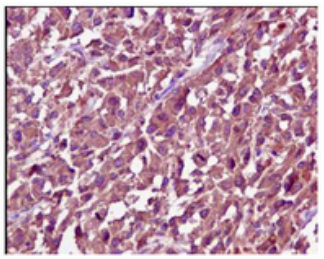
Staining for PNL-2 from Abcam



Staining of TRP-1 from Abcam. Clone:EPR21960



Staining of TRP-2/DCT from Abcam. Clone: EPR21986



Staining of Tyrosinase from Abcam. Clone: EPR10141

Continued on next page

Sun Exposure, Melanoma, and the Expanding Role of IHC (cont.)

UV Exposure and Melanoma Risk

Ultraviolet (UV) radiation damages the DNA in melanocytes. With prolonged exposure, this damage accumulates, especially in fair-skinned individuals, and may initiate malignant transformation. According to the American Cancer Society, melanoma accounts for roughly 1% of skin cancer cases but causes over 80% of skin cancer-related deaths. Early, accurate detection and molecular subtyping are critical, and IHC is a cornerstone of both.

Melanoma IHC

While S100 remains a go-to for its high sensitivity, the modern melanoma panel is far more nuanced. Here's a breakdown of classic, emerging, and veterinary-relevant markers you might encounter:

Classic Melanoma Markers

- **S100** – Sensitive but not specific; positive in nearly all melanomas, as well as nerve sheath tumors.
- **SOX10** – Nuclear marker, highly specific for neural crest–derived cells; more specific than S100.
- **HMB-45 (gp100)** – Targets immature melanosomes; helpful for identifying neoplastic vs benign lesions.
- **Melan-A (MART-1)** – Cytoplasmic stain, highly specific for melanocytes and melanoma cells.
- **MITF** – Transcription factor, nuclear stain, useful in identifying melanocytic origin, especially amelanotic lesions.
- **Ki-67** – Proliferation index often included to gauge aggressiveness.

Emerging & Specialized Markers

- **PNL2** – Highly sensitive and specific monoclonal antibody that reacts with melanocytic lesions, often more robust in decalcified or poorly fixed tissues than Melan-A or HMB-45. Performs well in both human and animal melanomas.
- **TRP-1** and **TRP-2** (Tyrosinase-Related Proteins) – Cytoplasmic markers that help confirm melanocytic origin, especially in amelanotic or desmoplastic melanomas.
- **Tyrosinase** – Used both diagnostically and therapeutically; also tested in molecular assays for minimal residual disease.

Histotech Tips for Melanoma Panels

- Use a panel-based approach. No single marker is definitive. Consider combining S100 or SOX10 (for sensitivity) with Melan-A, HMB-45, and TRP-2 (for specificity).
- Be aware of cytoplasmic vs nuclear staining patterns to interpret results correctly.
- Optimize antigen retrieval based on the clone: for example, PNL2 may require slightly different conditions than Melan-A.
- Melanoma markers can behave differently on decalcified or pigmented tissues, so include controls and document pre-analytical variables.

A Final Word

Whether you're cutting FFPE blocks from sun-damaged skin or running IHC on a suspected metastatic node, remember: your hands help make critical diagnostic decisions possible.

AUGUST 2025 ISSUE #2

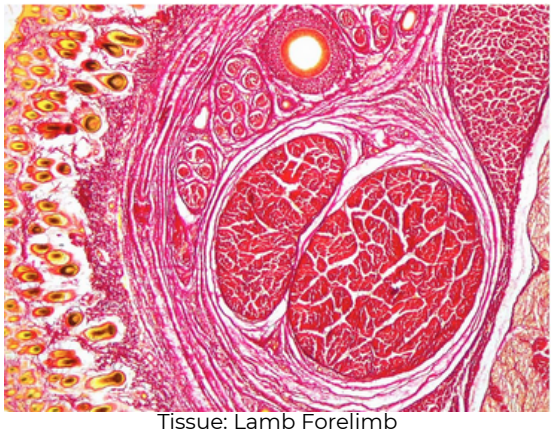
Stain of the Month
Picro-Sirius Red

This month we're highlighting a stain that's as beautiful as it is functional: Picro-Sirius Red. Used to visualize collagen fibers in tissue, this stain doesn't just pop under brightfield, it glows under polarized light, separating Type I and III collagen like a true tissue whisperer.

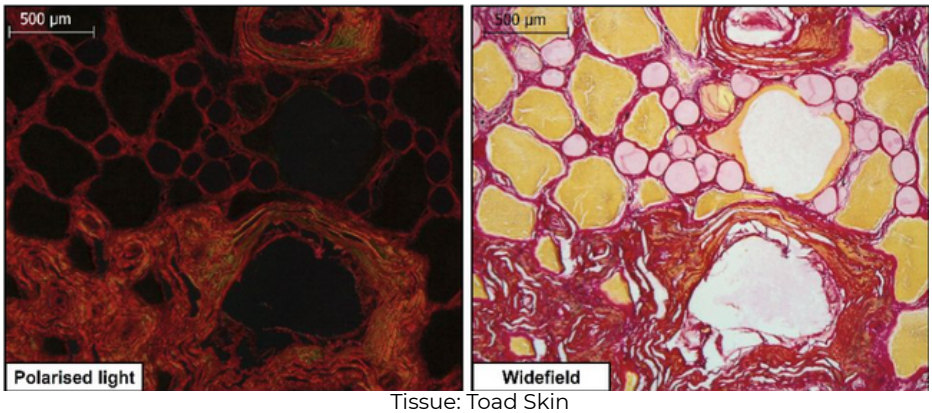
COLOR BREAKDOWN

Collagen Fiber Type	Birefringent Color	Notes
Type I Collagen	Bright Yellow Orange Red	Thicker fibers, strongly birefringent
Type III Collagen	Greenish	Thinner, less birefringent fibers

Note: The birefringence arises due to the alignment of the elongated Sirius Red dye molecules along collagen fibers, enhancing their natural birefringence.



Tissue: Lamb Forelimb
Staining: Hello Bio
Key: Collagen fibers- red; Muscle fibers- yellow.



Tissue: Toad Skin
Staining: Hello Bio
Key: Collagen fibers-red; Muscle fibers-yellow.
Under polarized light: Collagen 1 -orange/red; Collagen 3- green.

Reccomended Controls

Uterus
Red collagen bands in myometrium; birefringent

Fibrotic Liver
Collagen around portal triads, fibrous septa

Skin
Strong red staining in dermis; birefringent

Next Month: Feulgen DNA

Submit your stains to anlona@utmb.edu

AUGUST 2025 ISSUE #2

FREE CEUs!

Troubleshooting IHC

Presented by StatLab

Wednesday August 13th at 12:00pm CST

Register at https://www.statlab.com/webinars

HISTO MYTHBUSTERS

"You Can't Overfix Skin"

Busted! Yes, You Can! Here's Why It Matters

In the summer heat, skin biopsies start piling up, literally and figuratively. Whether you're working with punch biopsies for rashes or excisions for suspected melanoma, one myth still floats around labs:

"Skin is tough; you can't overfix it."

Wrong. You absolutely can overfix skin, and the consequences show up under the microscope (and in your IHC).

What Happens When You Overfix?

Prolonged exposure to 10% neutral buffered formalin causes:

- Cross-linking of proteins, which can mask antigenic sites, especially critical for IHC markers
- Hardening of dermal collagen, making microtomy a shoulder workout you didn't ask for.
- Epitope degradation, especially if fixation exceeds 72 hours in warm conditions.
- False negatives in IHC

What's the Fix for Fixation?

- Ideal fixation time for skin is 6 to 24 hours, depending on thickness.
- For large excisions or pigmented lesions, bread-loaf or bisect specimens to ensure full penetration.
- If you're not processing within 24–48 hours, transfer tissue to 70% ethanol to prevent overfixation while preserving morphology.

Tech Tip:
When working on melanoma panels, always document the fixation time, especially if some markers look weak. Antibody performance can vary drastically based on pre-analytic conditions.

Wanna bust another myth? Submit your suggestions to anlona@utmb.edu

AUGUST 2025 ISSUE #2

TIP OF THE MONTH

Melanoma Marker Mastery

Avoid False Negatives by Watching Your Retrieval!

Some melanoma markers, like HMB-45 and TRP-1/TRP-2, are extremely sensitive to over-retrieval, which can mask antigen sites or lead to nonspecific background staining.

Tip: Run your panel controls side-by-side with test tissue any time you adjust retrieval conditions, especially if you're rotating clones or switching vendors. What works beautifully for SOX10 might blow out PNL2.

Bonus: If you're getting weak or patchy Melan-A staining, try optimizing pH and retrieval time. Sometimes a milder citrate buffer (pH 6.0) gives better results than high pH EDTA, especially in veterinary or heavily pigmented tissues.

Histo-lol-gy

www.medlinkstudents.com

We all need a laugh sometimes...

etsy.com/shops/scientificgiftopia

AUGUST 2025 ISSUE #2

CALL FOR SUBMISSIONS!

Are you a passionate histotech with a cool case, clever tip, or compelling story to share? The Texas Society for Histotechnology (TSH) Newsletter wants to hear from YOU!

We're currently accepting submissions for upcoming issues and would love to feature your voice. Whether it's a how-to article on a staining technique, a fun lab anecdote, a photo of your latest beautiful slide, or a comic that only fellow histotechs will understand? There's a place for it in our pages.

You don't need to be a professional writer to contribute. We welcome pieces of all lengths and tones, from technical to humorous, and we're happy to help polish your draft if needed. Student voices and first-time contributors are especially encouraged to submit!
This is YOUR newsletter, let's make it a vibrant, collaborative space that reflects the creativity, expertise, and heart of our Texas histology community.

Submit your ideas, photos, or full articles to anlona@utmb.edu. Deadline for the next issue is August 25th.

Got something in your microtome drawer worth sharing? Don't keep it to yourself, submit it today!

JOIN TSH!

Click the link below to become a member of TSH and connect with a vibrant community of histology professionals across Texas. Whether you're a student, tech, pathologist, or vendor, there's a place for you in TSH! Gain access to exclusive resources, educational events, networking opportunities, and more. We'd' be glad to have ya! 🤠

👉 Join TSH here!

https://www.texashistology.com

The Texas Society of Histotechnology would like to thank our sponsors!

Leica BIOSYSTEMS

LUX MEDICAL LOGISTICS