Feb 13, 2023

Periodic acid Schiff hematoxylin (PASH) staining for human retina

DOI
dx.doi.org/10.17504/protocols.io.n2bvj8qzwgk5/v1

Dongfeng Cao[1], Jeffrey D. Messinger[1], Angela R.S. Kruse[1], Jamie Allen[1], David Anderson[1], Christine A. Curcio[1], Jeff Spraggins[1]

[1]Vanderbilt University

VU Biomolecular Multim... | Human BioMolecular Atl...

Angela R.S. Kruse
Vanderbilt University

DOI: https://dx.doi.org/10.17504/protocols.io.n2bvj8qzwgk5/v1

Protocol Citation: Dongfeng Cao, Jeffrey D. Messinger, Angela R.S. Kruse, Jamie Allen, David Anderson, Christine A. Curcio, Jeff Spraggins 2023. Periodic acid Schiff hematoxylin (PASH) staining for human retina. **protocols.io https://dx.doi.org/10.17504/protocols.io.n2bvj8qzwgk5/v1**

Protocol status: Working
We use this protocol and it's working

Created: February 13, 2023

Last Modified: October 18, 2023

Protocol Integer ID: 76915

Keywords: histology, PASH, retina, stain, human retina periodic acid schiff hematoxylin, periodic acid schiff hematoxylin, retinal pigment, retinal pigment epithelium, carbohydrate in human retinal section, human retina, clinical retina imaging, µm thick cryosections of human retina, human retinal section, staining protocol, comparison to clinical retina imaging, retinal pigment epithelial expression of complement regulator cd46, glycogen in liver tissue, modified harris hematoxylin, degeneration in the macular region, macular degeneration, nucleic acid, nucleic acids in nuclei, demonstrating glycogen, harris hematoxylin, related macular degeneration, american journal of ophthalmology, senile disciform macular degeneration, exp eye re, histochemistry, hematoxylin, containing abundant lipofuscin, glycogen, macular region, ophthalmology

Funders Acknowledgements:
HuBMAP
Grant ID: NIH U54 EY032442

Abstract

Periodic acid Schiff hematoxylin (PASH) staining protocol is adapted from other tissues to identify carbohydrate in human retinal sections in our lab. This method is used for labeling 10-12 µm thick cryosections of human retina, RPE, choroid, and sclera, preserved within 6 hours of death in 4% paraformaldehyde in 0.1 M phosphate buffer, picked up on SuperFrost glass slides, and stored at -80°C. It is intended to be counterstained for nucleic acids in nuclei using modified Harris hematoxylin.

The most common application is for demonstrating glycogen in liver tissue. Positive staining for glycogen is magenta, and nuclei stained by hematoxylin are blue.

This PASH staining protocol is helpful to identify drusen, basal laminar deposits, retinal pigment epithelium (RPE, containing abundant lipofuscin), nuclei, and cell layers of the retina, choroid, and sclera. It is preferable to traditional H&E staining for structures and pathology specific to age related macular degeneration (AMD). It is particularly useful for diagnosis and comparison to clinical retina imaging, especially optical coherence tomography. Since the late 1960s, PASH applied to human AMD specimens was noted to stain an eosinophilic material at the base of the RPE (now known as basal laminar deposit), drusen (the characteristic lesions), and Bruch's membrane. [1-4]

1.Gass JDM. Pathogenesis of disciform detachment of the neuroepithelium. III. Senile disciform macular degeneration. *Am. J. Ophthalmol.* 1967;63:617-644. PMID 6019308
2.Farkas TG, Sylvester V, Archer D, Altona M. The histochemistry of drusen. *American Journal of Ophthalmology.* 1971;71(6):1206-1215. PMID 4253686
3.Sarks SH. Ageing and degeneration in the macular region: a clinico-pathological study. *Br. J. Ophthalmol.* 1976;60(5):324-341. PMID 952802
4.Vogt SD, Curcio CA, Wang L, Li C-M, McGwin G, Jr, Medeiros NE, Philp NJ, Kimble JA, Read RW. Retinal pigment epithelial expression of complement regulator CD46 is altered early in the course of geographic atrophy. *Exp Eye Res.* 2011;93(4):413-423 PMID 21684273

Materials

PASH kit (Poly Scientific R&D Corp.Bay Shore, NY, Cat# K047)

Slide warmer (Fisher, Cat# 120594)
De-ionized (DI) water
Ethanol (many vendors)
Xylenes (many vendors)
Permount (EMS, cat#17986-01)

Troubleshooting

Safety warnings

All steps involving xylenes must be performed in a fume hood

PASH Staining

1h 51m

1 Remove glass slides with human retinal cryosections from -80 °C, keep on ice during transfer from freezer to bench.

2 Put glass slides on slide warmer (Fisher, Cat# 120594) at 37 °C overnight to dehydrate.

3 Label slides with PASH, date, and additional relevant information.

4 Hydrate slides with de-ionized (DI) water for 00:05:00 — 5m

5 Incubate with 0.5% Periodic Acid from kit for 00:05:00 — 5m

6 Rinse DI water for 00:05:00 — 5m

7 Incubate with Schiff Reagent from kit for 00:15:00 — 15m

8 Rinse with DI water for 00:10:00 — 10m

9 Incubate with Harris Hematoxylin from kit for 00:05:00 — 5m

10 Rinse with DI water for 00:05:00 — 5m

11 Quick dip in 0.5% Acid Alcohol from kit (or 00:00:20) — 20s

12 Rinse with DI water for 00:05:00 — 5m

13 Quick 2 dips in Bluing solution of 1% Lithium Carbonate (or 00:00:40) 40s

14 Rinse with DI water for 00:05:00 5m

15 Dehydrate using the following series: 50m
75% ethanol 00:05:00
75% ethanol 00:05:00
85% ethanol 00:05:00
85% ethanol 00:05:00
95% ethanol 00:05:00
95% ethanol 00:05:00
100% ethanol 00:05:00
100% ethanol 00:05:00
Xylenes 00:05:00
Xylenes 00:05:00

16 Mount with Permount (EMS, cat#17986-01) and air dry in hood for overnight.

17 Image using Virtual Slide System (OLYMPUS, Japan) using 20x, 40, and 60x objectives.

18 Save images as tiff files with clear labeling: Eye ID/L, R_Age/Gender_Slide #_Dye_magnification (e.g.: 1234567L_97F_050_PASH).

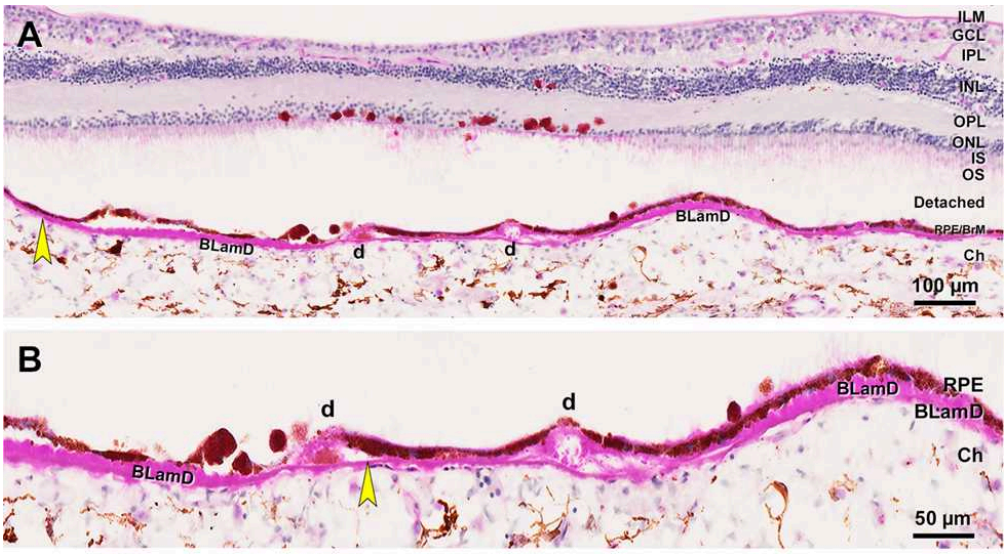

Figure 1. Periodic acid Schiff hematoxylin (PASH) staining human retina. Yellow arrowhead, Bruch's membrane (BrM); d, drusen, BLamD, basal laminar deposits; scale bars labels for each panel. (**A**) PASH reveal the structure of human retina by each layer and also labels drusen, basal laminar deposits (BLamD), retinal pigment epithelium (RPE) and Bruch's membrane (BrM). (**B**) Higher magnification of drusen and BLamD regions of the retina in A. ILM, internal limiting membrane; GCL, Ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; IS, inner segments of photoreceptors; OS, outer segments of photoreceptors; Detached, artifact detached between retina and RPE-BrM-Choroid; RPE, retinal pigment epithelium; BrM, Bruch's membrane; Ch, choroid. Image: 1234567L-92M-040-PASH-40x

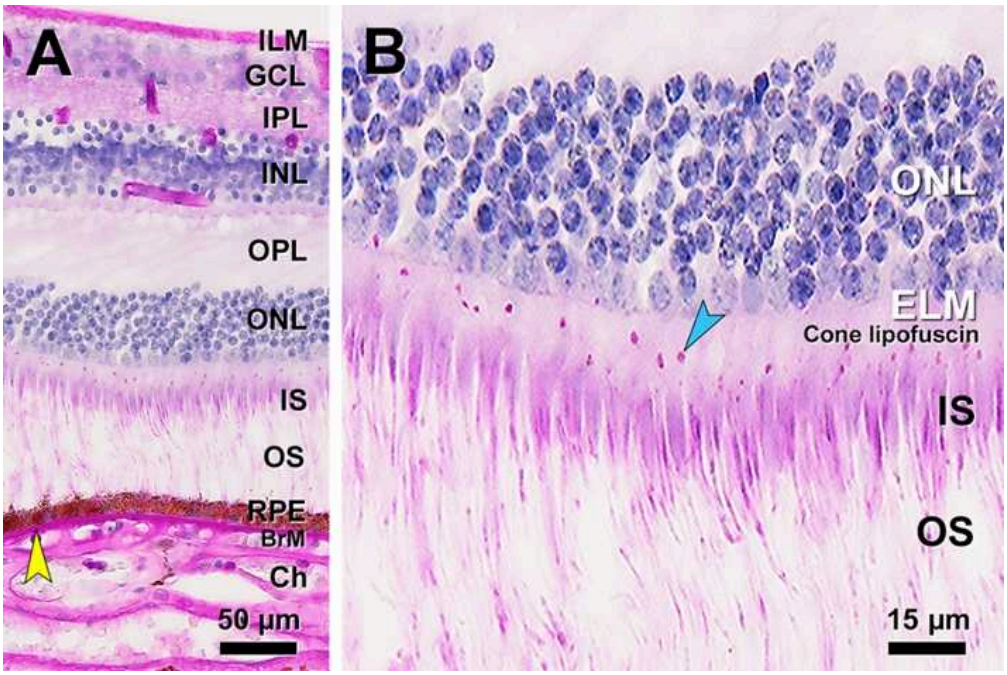

Figure 2. Periodic acid Schiff hematoxylin (PASH) staining lipofuscin in cone photoreceptors of human retina. Yellow arrowhead, Bruch's membrane (BrM); cyan arrowhead, cone lipofuscin; scale bars labels for each panel. (**A**) PASH staining reveals the structure of human retina by each layer. (**B**) Higher magnification shows lipofuscin in the myoid part of cone inner segments and a few in the ONL. These organelles are found in aged normal and AMD eyes [*5*]. ILM, internal limiting membrane; GCL, Ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; IS, inner segments of photoreceptors; OS, outer segments of photoreceptors; Detached, artifact detached between retina and RPE-BrM-Choroid; RPE, retinal pigment epithelium; BrM, Bruch's membrane; Ch, choroid. Image: 1234567L-97F-043-PASH-40x

5.Echols BS, Clark ME, Swain TA, Chen L, Kar D, Zhang Y, Sloan KR, McGwin Jr G, Singireddy R, Mays C, Kilpatrick D, Crosson JN, Owsley C, Curcio CA. Hyperreflective foci and specks are associated with delayed rod-mediated dark adaptation in non-neovascular age-related macular degeneration. *Ophthalmology Retina.* 2020;4(11):1059-1068. PMID 32389889