Objectives
2014 LID-PEG JHU Training Workshop
Project 3 & Skills Development Training Core (B)
The Johns Hopkins University School of Medicine, Wood Basic Science Building (WBSB)
725 N. Wolfe Street, Baltimore, MD 21205

Module 1: Agarose-Acrylamide Composite Gels; mucin and ganglioside extractions

a. Learn how to prepare composite agarose-acrylamide gels for the separation of high molecular weight (>500kDa) proteins.

b. Learn how to prepare high molecular weight proteins extracted from different compartments of the human lung for siglec counter-receptor activity.

c. Use agarose-acrylamide gels, to enhance resolution, rigidity and transfer of high molecular weight proteins.

d. Learn how to extract and purify gangliosides from mouse brains.

Module 2: Lectin blotting

a. Develop siglec overlay blots to identify siglec counter-receptor species from different lung compartments resolved on composite agarose-acrylamide gels.

b. Use quantitative siglec dot blotting to determine the relative abundance of siglec counter-receptors from human lung.

Module 3: Glycolipid and neoglycolipid ELISA and ganglioside thin-layer chromatography

a. Learn how to prepare glycolipid-adsorbed multi-well dishes for lectin probing

b. Test neoglycolipids and gangliosides for siglec binding specificity.

c. Learn how to resolve and detect gangliosides using thin layer chromatography (TLC).

Module 4: Lectin histochemistry

a. Learn how to use of polyvalent lectins to detect counter-receptors in fixed tissues.

b. Determine the distribution of siglec counter-receptors in human lung.

9th June	All Groups
8:00	Transport hotel to JHUSM
8:30 - 9:30	1. Welcome, Introductions, Groups 2. Make composite Gels
9:30 - 12:00	1. Extract tracheal epithelium in detergent 2. Pulverize remaining trachea; GuHCl extract 3. Pulverize lung parencyma; detegent extract 4. Composite Gel Electrophoresis
12:30 - 2:00	Box Lunch Mucins Lecture & Discussion (Dr. Schnaar)
2:00 - 3:00	1. Blot composite gels 2. Block; incubate blots with siglecs overnight
3:00 - 5:00	Extract & purify mouse brain ganglisides
5:00 - 5:30	Recap and transport back to hotel

10th June	Group1	Group2	Group 3
8:00	Transport hotel to JHUSM		
8:30 - 12:00	1. Process GuHCl extract, dilute, load dot blot 2. Wash, process, and image siglec blots 3. Process, stain, image, quantify dot blots	1. Prepare lipid-adsorbed plates for ELISA 2. Subject brain gangliosides to TLC 3. Lectin probe lipid ELISA plates	1. Siglec-COMP stain lung tissue sections 2. Develop stained sections with anti-His Ab 3. Develop anti-His with Vector Red stain 4. Dry, mount, image
12:30 - 2:00	Box Lunch & Glycolipid Lecture & Discussion by Dr. Schnaar		
2:00 - 5:30	1. Prepare lipid-adsorbed plates for ELISA 2. Subject brain gangliosides to TLC 3. Lectin probe lipid ELISA plates	1. Siglec-COMP stain lung tissue sections 2. Develop stained sections with anti-His Ab 3. Develop anti-His with Vector Red stain 4. Dry, mount, image	1. Process GuHCl extract, dilute, load dot blot 2. Wash, process, and image siglec blots 3. Process, stain, image, quantify dot blots
6:30 - 10:00	Social Event - Baltimore Orioles vs Boston Red Sox		

11th June	Group1	Group2	Group 3
8:30 - 12:00	1. Siglec-COMP stain lung tissue sections 2. Develop stained sections with anti-His Ab 3. Develop anti-His with Vector Red stain 4. Dry, mount, image	1. Process GuHCl extract, dilute, load dot blot 2. Wash, process, and image siglec blots 3. Process, stain, image, quantify dot blots	1. Prepare lipid-adsorbed plates for ELISA 2. Subject brain gangliosides to TLC 3. Lectin probe lipid ELISA plates
12:30 - 2:00	Wrap-up LUNCH		