3D Reconstructed Human Airway Models: Effect of Acclimation Conditions on Biomarker and Inflammatory Response Following Tissue Challenge

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Institute for In Vitro Sciences, Inc. (IIVS)

1. **Science** - Practical Knowledge
2. **Education** - Dissemination of Information
3. **Outreach** - Advocacy for the Methods

Assessment of In Vitro COPD Models for Tobacco Regulatory Science

December 8-10, 2014

Bethesda, MD

Supported by FDA-CTP R13 grant

Technical Workshop:

"In Vitro Models for Goblet Cell Hyperplasia, Mucus Production, and Ciliary Beating Assays"

June 16-18, 2015
Gaithersburg, MD

- IIVS has had substantial involvement in numerous validation studies or method evaluations (ECVAM, ICCVAM, OECD expert groups)

Institute for In Vitro Sciences, Inc. (IIVS)

Respiratory Toxicology Program:

- Developed to meet the growing demand for the assessment of inhaled or other pulmonary toxicants:
  - Household products, personal care products, fragrances, pharmaceuticals

  …and now tobacco products such as MRTPs (e.g. E-cigarette aerosols)

http://vapelifemagazine.com/wp-content/uploads/2015/03/vape-bar-610x250.jpg

- Whenever possible, we work exclusively with *in vitro* models of human tissue origin

- Experience with monolayer cultures, but also with complex 3D models such as precision-cut lung slices and reconstructed airways grown at the air-liquid interface (ALI)

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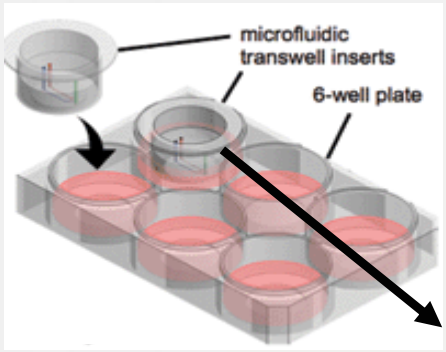
IIVS' Respiratory Toxicology Program

Human reconstructed 3D models (e.g. EpiAirway™ or MucilAir™)

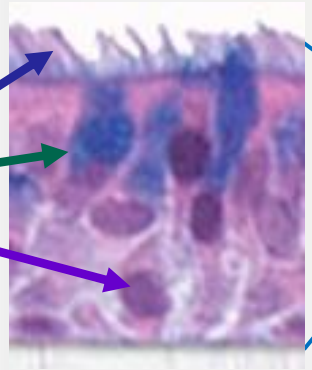


Cells:

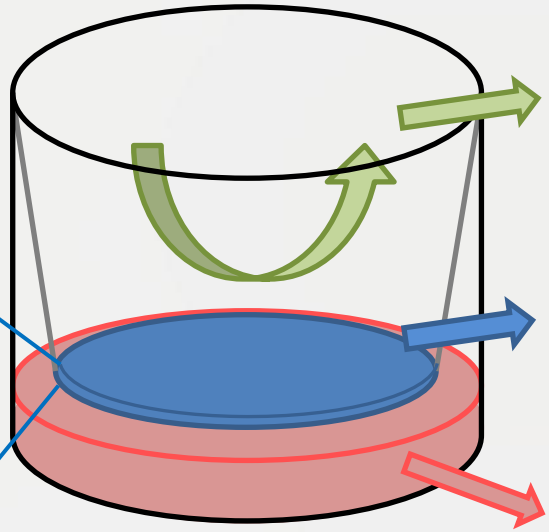
Ciliated

Goblet

Basal



Microphotograph source:
http://www.epithelix.com/products/mucilair



Tissue insert at air liquid interface (ALI)

Apical Rinse (lavage fluid)
- Inhalation exposures
- Surfactant changes
- Leakage/signaling marker responses (LDH, cytokines, chemokines)

Lysate (tissue)
- Tissue responses (multicellular)
- -omics, biomarker regulation
- Histology – specialty stains, morphology changes

Medium (blood)
- Systemic exposures
- Leakage/signaling marker responses (LDH, cytokines, chemokines)

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Why EpiAirway Characterization?

- Achieve better understanding of tissues
- Test variability across tissues
- Inconsistent response to reference chemicals

E.g. baseline values with a heat map of values – high variability

Medium IL-8
(all untreated)

@24hr			@48hr
12867	13637	13252	10873
13173	10014	11593	15131
15457	9930	12694	12230
12989	19292	16140	20980
16855	11671	14263	17550
6514	11089	8801	12697
7248	5458	6353	9097
9545	8397	8971	8966
9627	9986	9807	

Medium IL-6
(untreated)
@48hr

197
244
473
146

Study Objectives

1. Evaluate **marker variability** across replicate tissues and the effect of **normalizing** to protein

2. Assess **acclimation**: impact on tissue response
   a) **Inclusion of anti-inflammatory agent** (2 µM hydrocortisone; HC)
   b) **24 vs 48hr** acclimation duration

3. Expose tissue to two **pulmonary toxicants** and assess response by **sampling all compartments**
   a) Lipopolysaccharide (LPS) at 5 µg/mL
   b) Poly I:C (Polyinosinic:polycytidylic acid; viral simulator) at 15 µg/mL

1. Marker Variability and Normalization

TEST ARTICLE	Test Number	Type	protein (ng/mL) 1	2	3	Average	S.D.
Neg. Ctrl HC+	2	BCA	656	636	1273	**855**	362

IL-6	24hr	2	Media	128.2	78.9	76.2	**94.5**	29.3
	48hr	2	Media	61.3	41.2	48.6	**50.4**	10.2
	24hr	2	Apical	57.8	39.8	27.8	**41.8**	15.1
	48hr	2	Apical	51.8	44.8	33.8	**43.4**	9.1
	48hr	2	Lysis	15.9	10.6	11.9	**12.8**	2.8

IP-10	24hr	2	Media	169.7	112.7	244.8	**175.7**	66.2
	48hr	2	Media	134.1	23.9	66.5	**74.8**	55.5
	24hr	2	Apical	153.1	41.2	73.1	**89.1**	57.6
	48hr	2	Apical	195.8	131.7	105.1	**144.2**	46.6
	48hr	2	Lysis	176.9	2.7	-15.0	**54.8**	106.1

IL-6 and IP-10 values are pg/mL

- Tissue protein values matched expected values, but had up to 2-fold differences across inserts
- Protein levels did not correspond with baseline or induced marker values in any compartment

1. Marker Variability and Normalization

E.g. "rogue" responses following exposure

	Poly I:C (++-) HC				
	IL-6 N = 1	2	3		
0hr	Apical	10	18	8	**12**	5
24hr	Dosed/Apical	971	185	180	**445**	455
24hr	Lysis	51	20	19	**30**	18
0hr	Media	13	45	36	**31**	16
24hr	Dosed/Media	197	97	92	**129**	59
	IL-8					
0hr	Apical	1142	2177	1088	**1469**	614
24hr	Dosed/Apical	21748	20357	19458	**20521**	1154
24hr	Lysis	2948	2690	2126	**2588**	421
0hr	Media	4493	7302	7571	**6455**	1705
24hr	Dosed/Media	21403	25839	24197	**23813**	2243

Protein content (ng/mL)

BCA	**918**	**646**	**745**

Disparity in marker values not consistent across markers in replicate groups

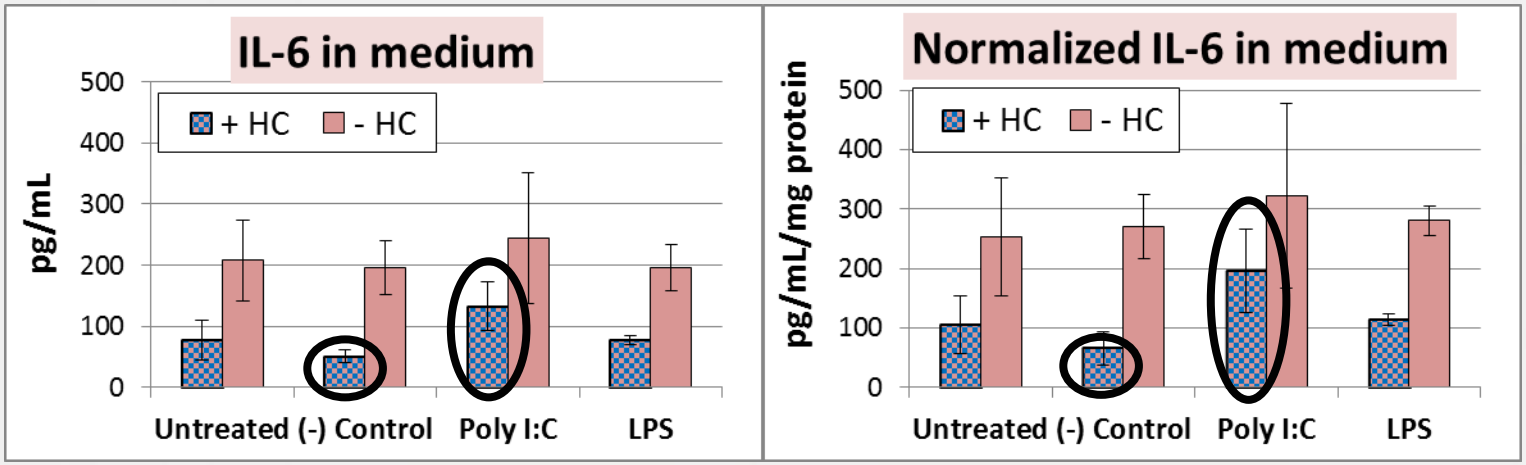
Rep #1 has much higher IL-6 values than the remaining 2 reps in its group (post exposure) baseline values don't reflect this

For IL-8, no substantial difference is seen across replicates

Cannot use a pretreated tissue baseline as reference

1. Marker Variability and Normalization

Protein Normalization: possible increase in relative responses, but also CV

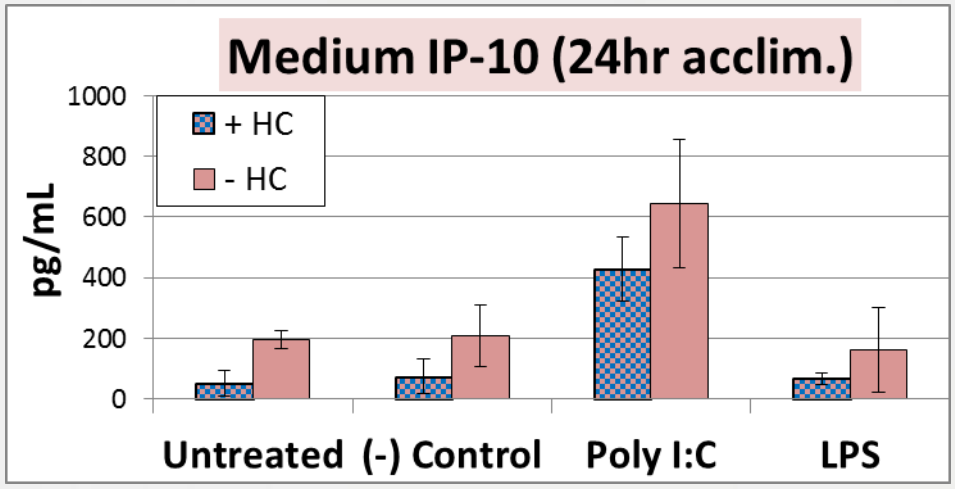


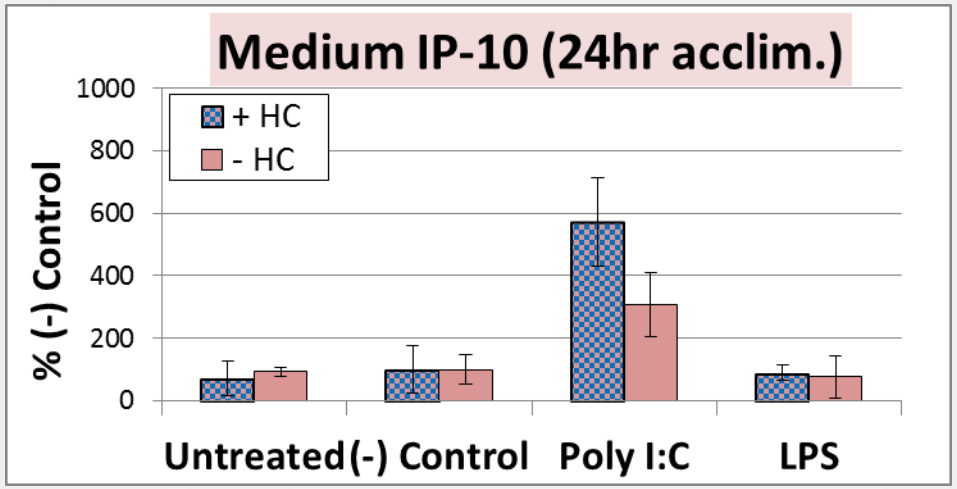
24hr acclim.

note response ratios

- The same trend was seen for the 48hr acclimation group
- All acclimation paradigms showed same effect and it was apparent for all biomarkers sampled
- Normalization diminished significant differences of treated groups vs controls

1. Assess acclimation: Inclusion of HC

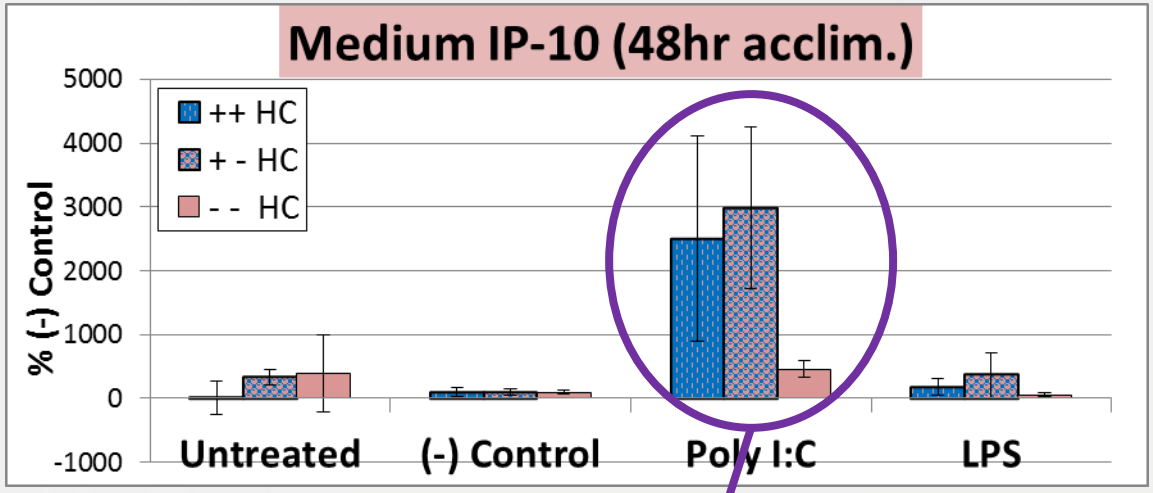


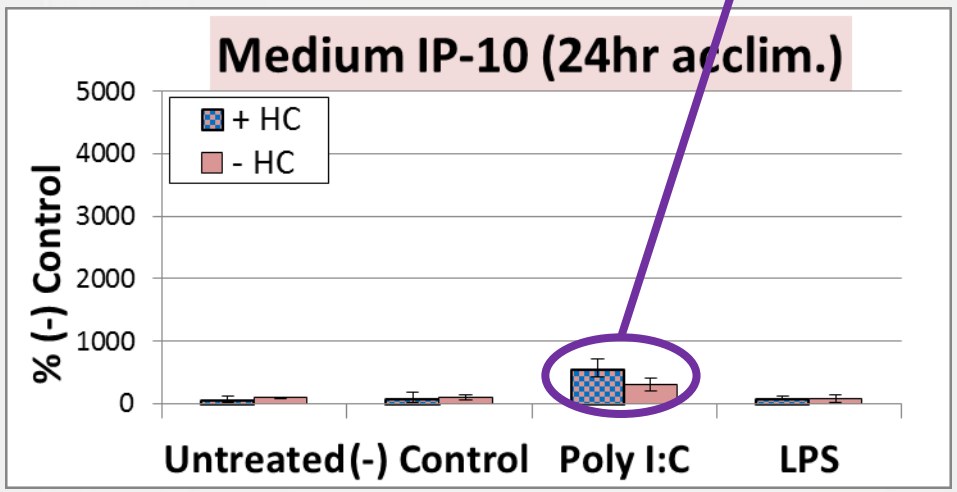


- Omitting HC can result in a greater signal, **but** baseline values are also higher
- Normalizing to (-) control group shows HC inclusive groups to have greater signal over control

Conclusion: Inclusion of HC lowers IP-10 baseline levels & elicits greater response over (-) control. This was consistently seen across markers assayed.

1. Assess acclimation: 24hr vs 48hr



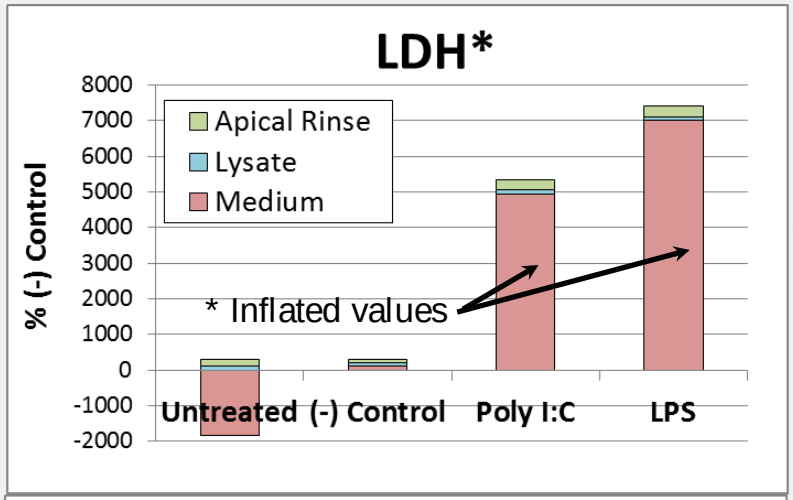


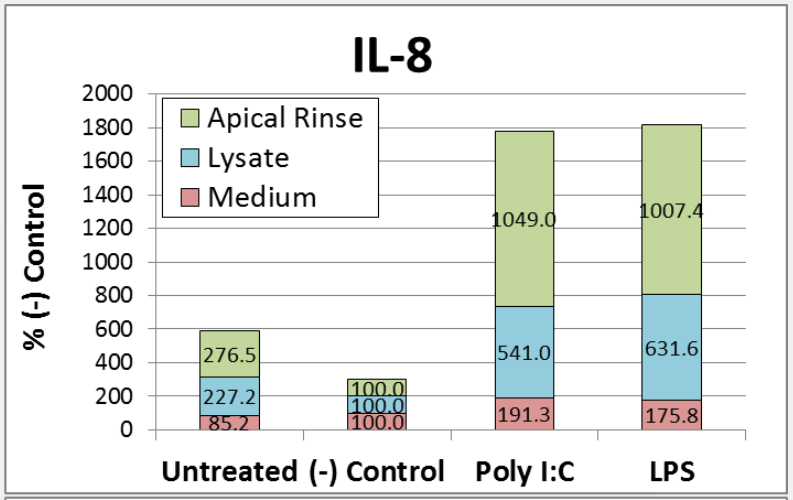
- 48hr acclimation also shows HC inclusive groups having greatest response
- Scaling the 24hr acclimation as above allows a direct comparison of relative response

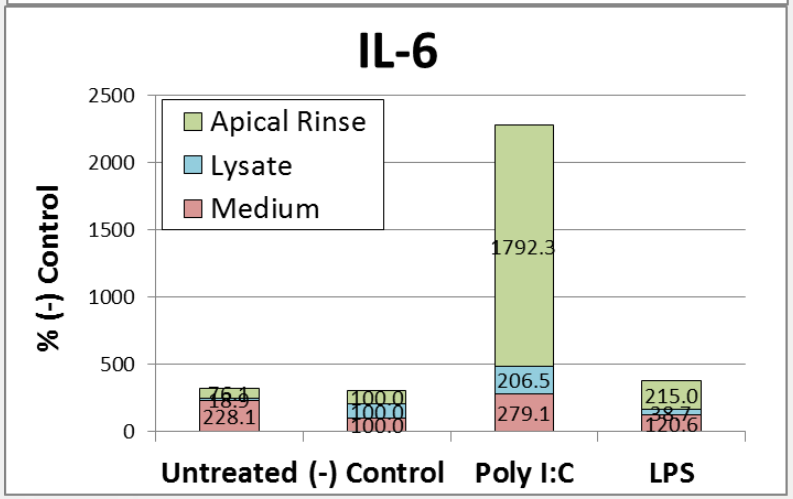
Conclusion: 48hr acclimation typically resulted in a substantially greater response over control. This was consistently seen across markers assayed.

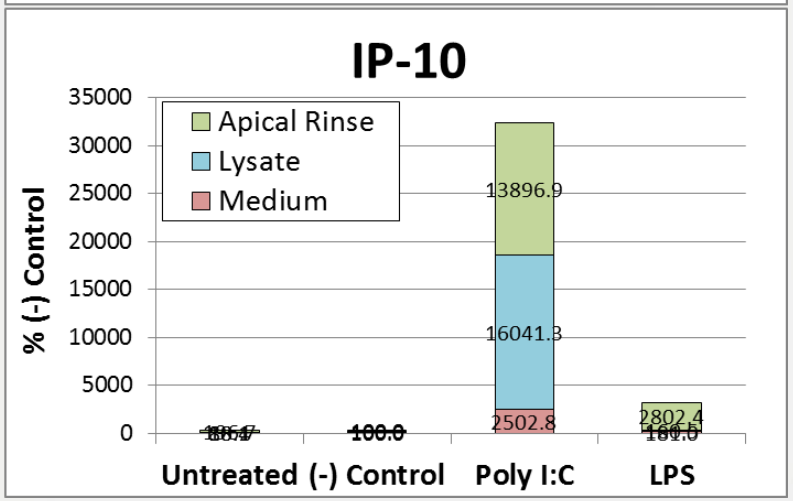
2. Expose tissue to two pulmonary toxicants and assess response by sampling all compartments

Biomarker results of the 48hr HC inclusion group , all expressed as % of (-) Control





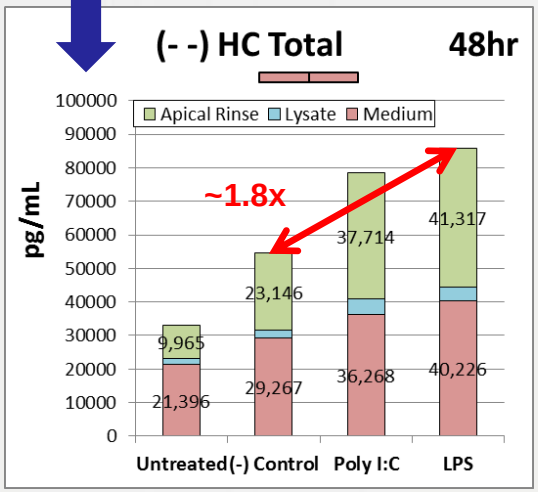


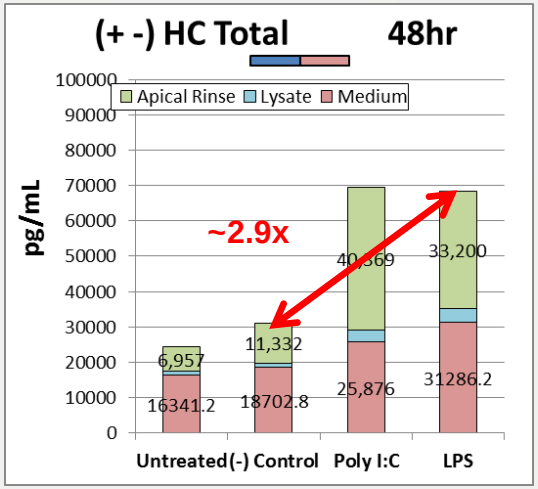


* Medium LDH not evaluable as % Control due to very low baseline values

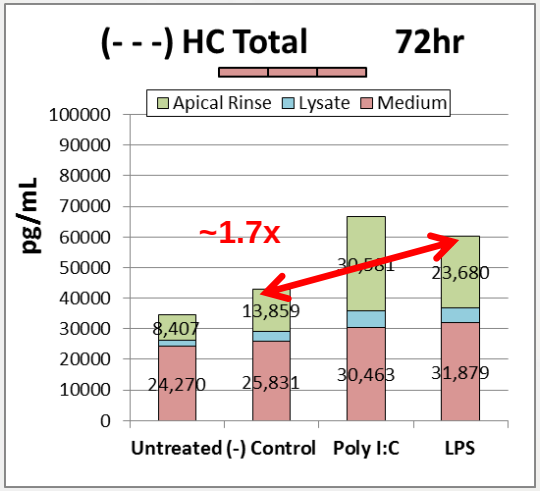
2. Expose tissue to two pulmonary toxicants and assess response by sampling all compartments

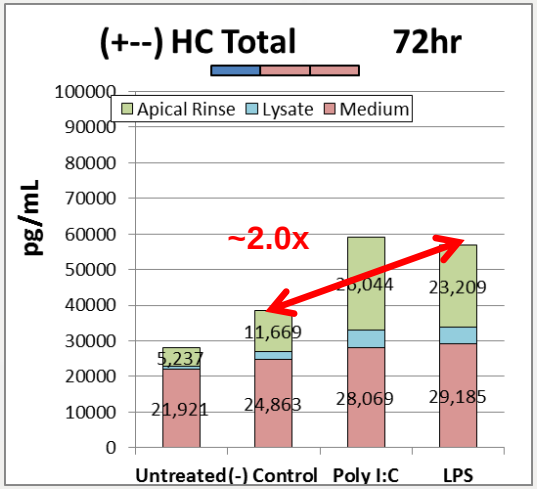
(--) HC group had greatest cumulative IL-8 levels, but also high baseline control

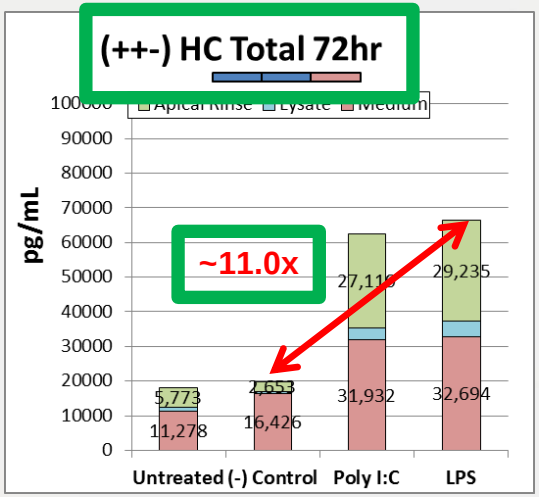




A comparison of apical rinse LPS-induced marker increases, across all acclimation paradigms illustrates how HC inclusion and extra time results in greater response over control







2. Expose tissue to two pulmonary toxicants and assess response by sampling all compartments

Fold Change over Negative Control (all 48hr HC groups)

- All data normalized to sampling volume

	LDH		IL-6		IL-8		IP-10			
	Poly I:C	LPS	Poly I:C	LPS	Poly I:C	LPS	Poly I:C	LPS	**AVE**	SD
Apical Wash	2.8	2.9	17.9	2.1	10.5	10.1	139.0	28.0	**26.7**	46.2
Lysate	1.1	1.1	3.2	1.3	5.4	6.3	160.4	1.6	**22.6**	55.7
Medium	NE*	NE*	1.3	1.2	1.9	1.8	25.0	1.8	**5.5**	9.6

* Not Evaluable - baseline values very low

- Apical compartment is always yields greater marker quantities than basal (medium) compartment
- Tissue lysate typically also has greater values, but can be used for histology

NOTE: both Poly I:C and LPS demonstrate distinct marker expression patterns in the apical compartment but not necessarily in the medium

General Conclusions

- Variability of marker levels was pervasive in this pilot study
  - Protein normalization nor the use of a tissue-specific pretreatment baseline mitigated variability or establishing difference from controls
- The 48hr acclimation period, with the inclusion of HC produced greater responses over control to tissue challenge
- The apical compartment consistently yielded high marker values, much greater than those found in medium

Question:

1. Are greater marker quantities in apical compartment due to exposure location??
2. Should we expect compound-specific marker "signatures" when obtaining datasets for multiple markers such as inflammatory mediators

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Recommendations for Model Use

Methods:

- Acclimate for 48hr (or longer?) and include an anti-inflammatory agent until ready for use

- Consider Apical wash a good candidate for detecting biomarker change
  - Look for "signatures" that may help differentiate chemicals or products

- The use of tissue itself would be better suited to examine histological changes
  - Protein content (or another marker – e.g. total DNA) is unlikely to be helpful

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Contact IIVS for more Information!

Questions?

Workshop Report:

"In Vitro COPD Models for Tobacco Regulatory science"

Upcoming IIVS workshop:

"In Vitro Exposure Systems and Dosimetry Assessment Tools for Inhaled Tobacco Products" April 4-8, 2016 Bethesda, MD

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