

SENSING LUNG INJURY VIA EXHALED BREATH CONDENSATE

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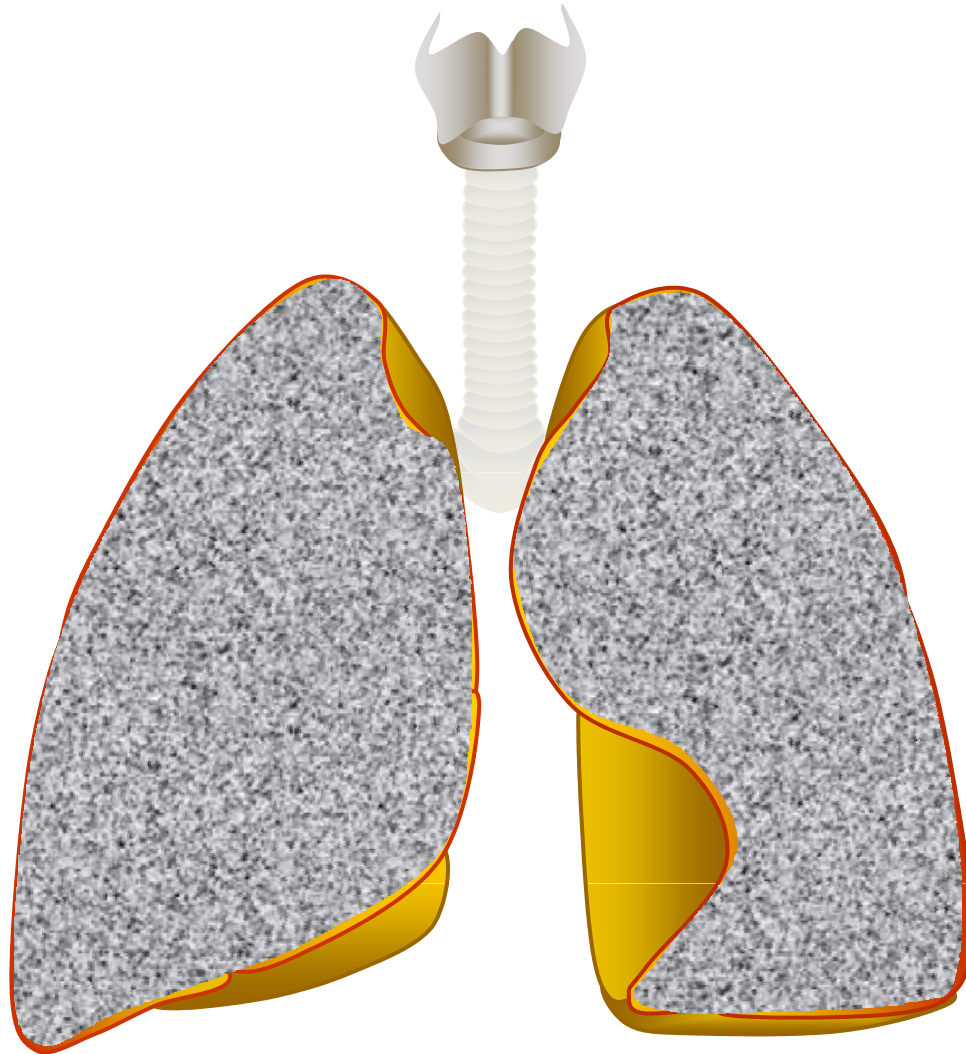
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**INNOVATION FORUM
MAY 14th 2013**

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The Lung is the Only Internal Organ that is in Constant Contact with the Environment



Does the Air we Breathe Out Reflect our “Inside” Environment



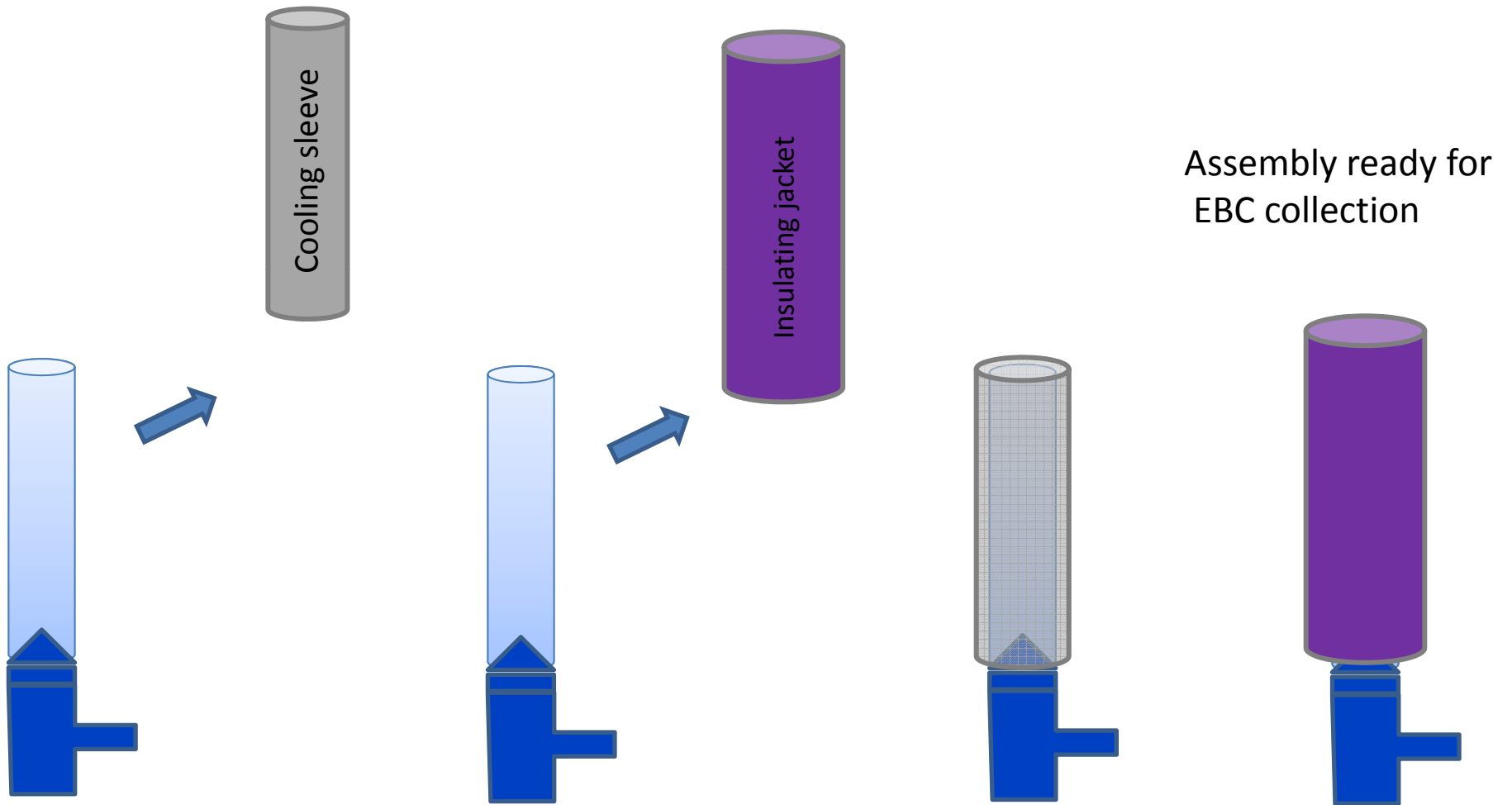
Does Exhaled Breath Contain Proteins or Other Markers that Reflect the Microenvironment in the Lung



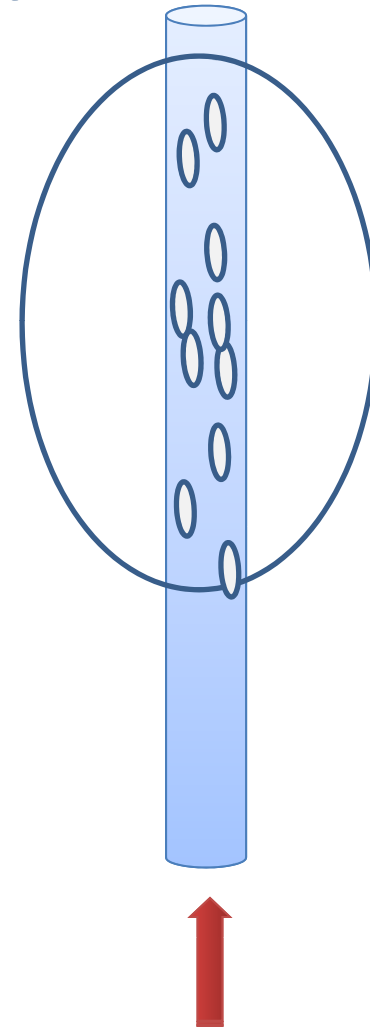
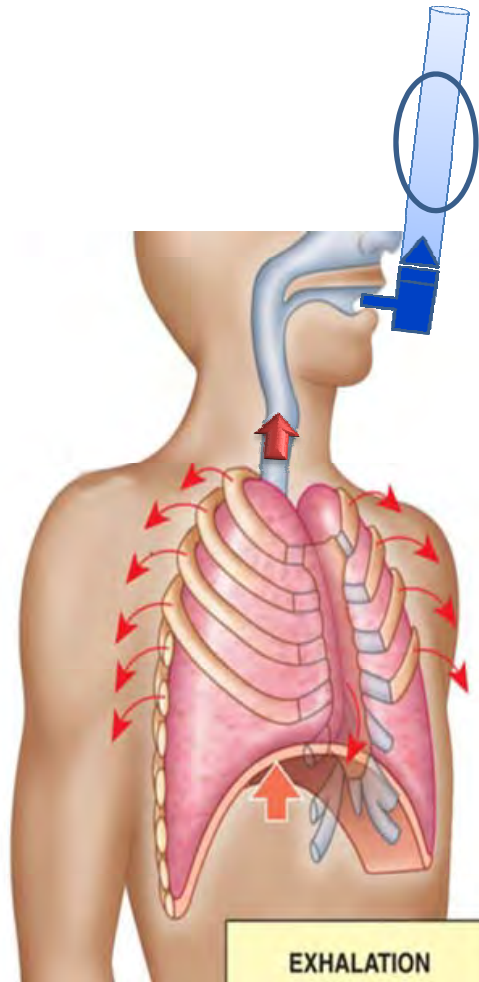
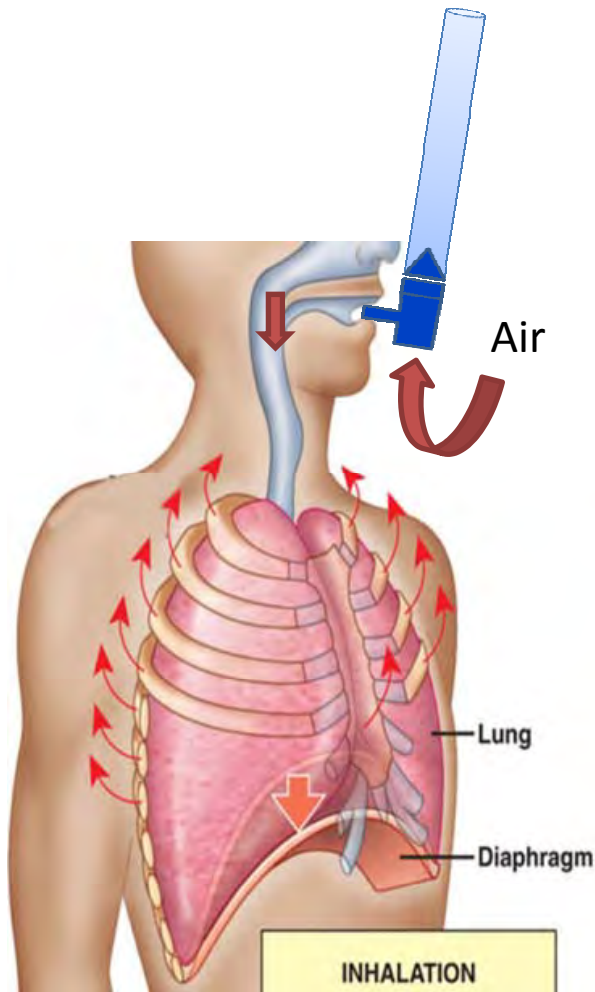
Hypothesis

- People from an **urban** area demonstrate perturbations in their lung microenvironment as measured by MS proteomics on exhaled breath condensate compared to people from a **rural** area

Collecting Exhaled Breath Condensate



The EBC is Collected and Analysed in The Laboratory



Collecting Exhaled Breath Far More Comfortable than Drawing Blood



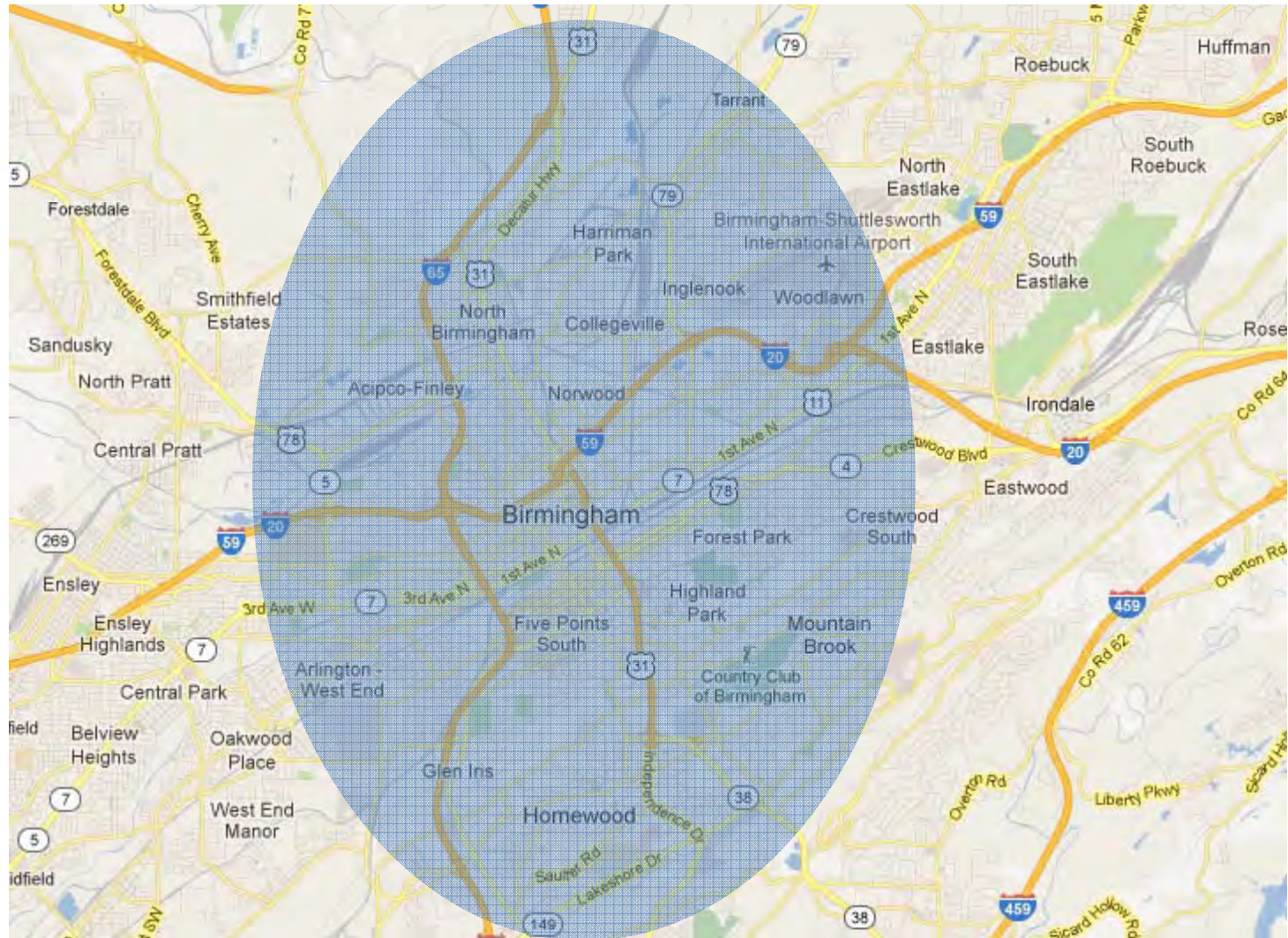
Sample Collection



Urban



Rural



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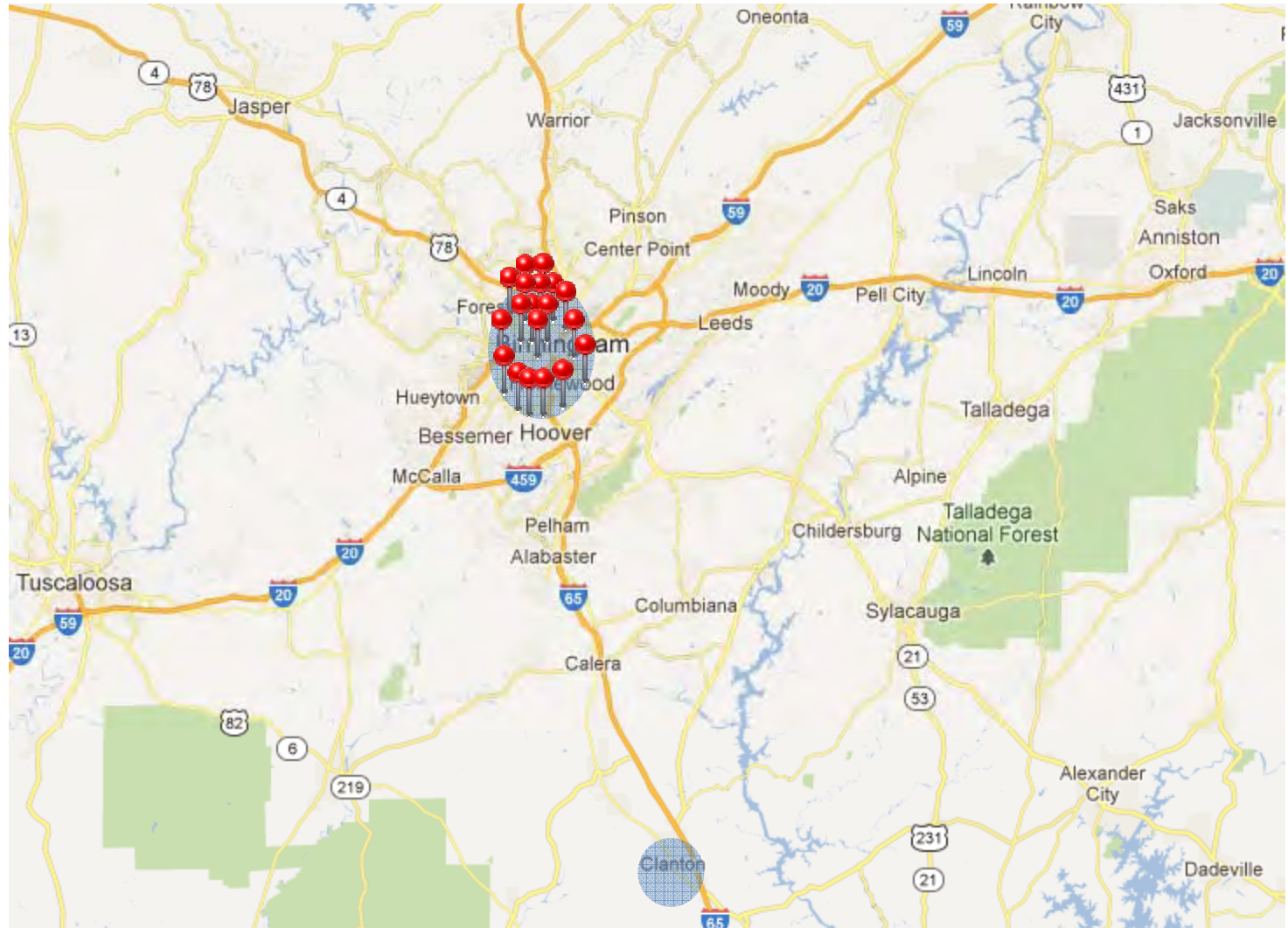
Sample Collection



Urban



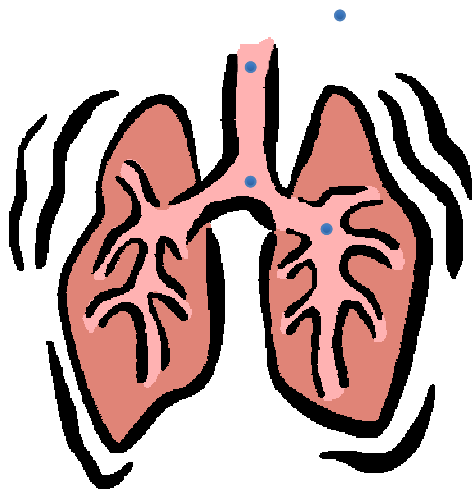
Rural



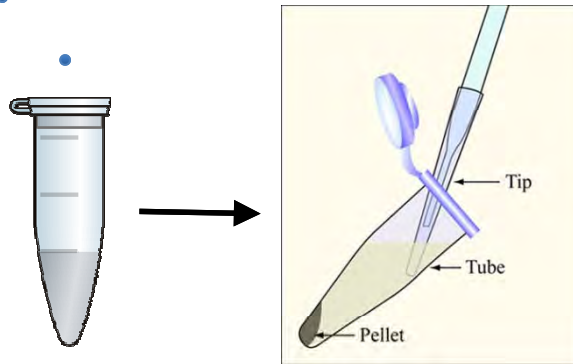
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Samples brought to laboratory and
subjected to Mass Spectroscopy.

What is Mass Spectroscopy?

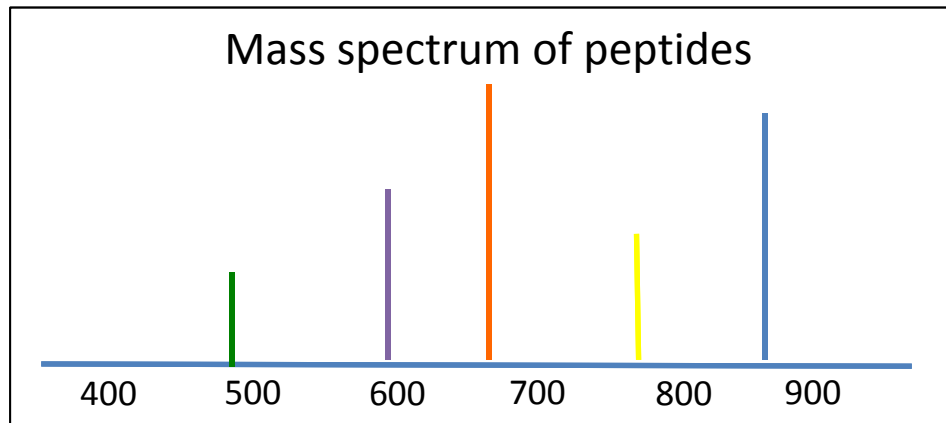
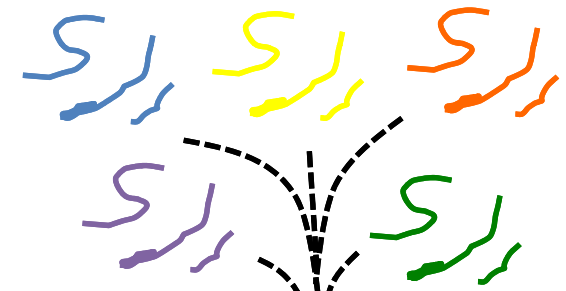
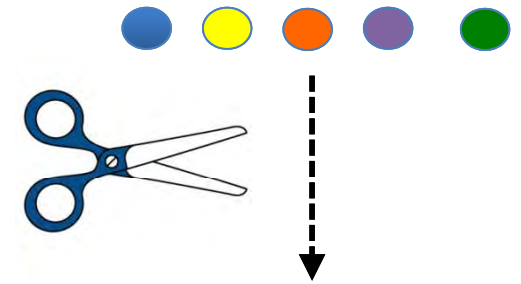


Emerging droplets are sampling events occurring in the lung



Precipitating the proteins

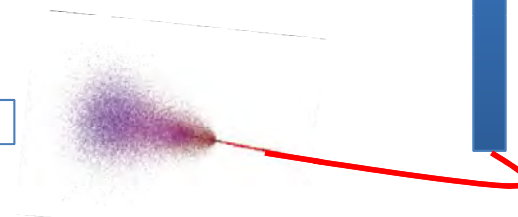
Breath condensate proteins

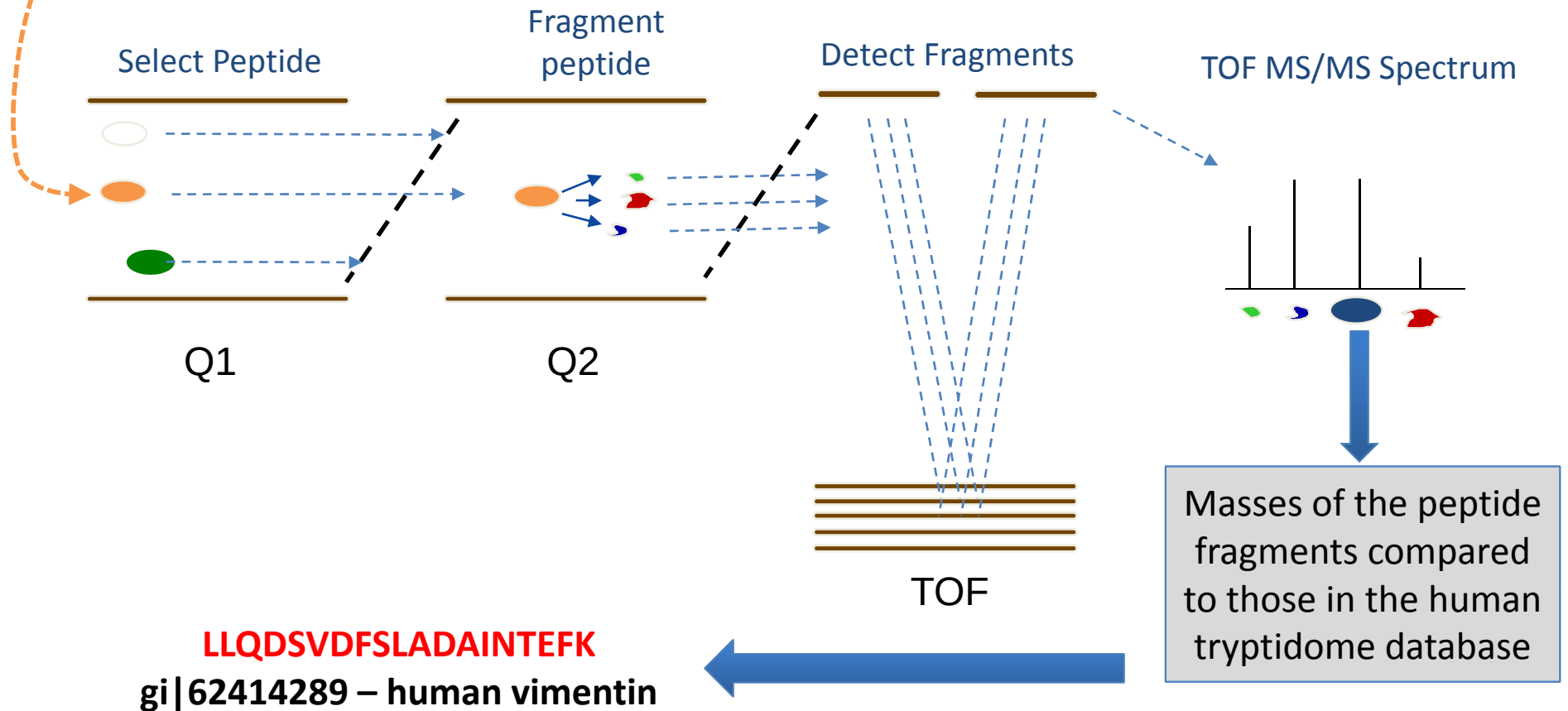
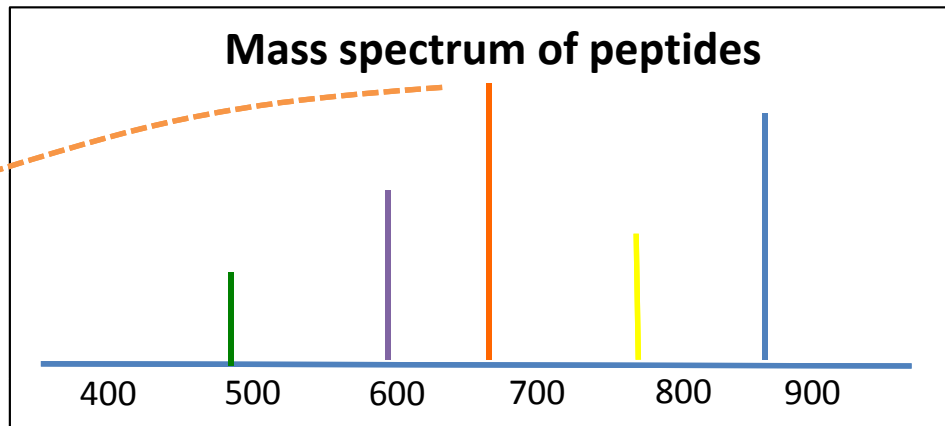


Convert time to mass



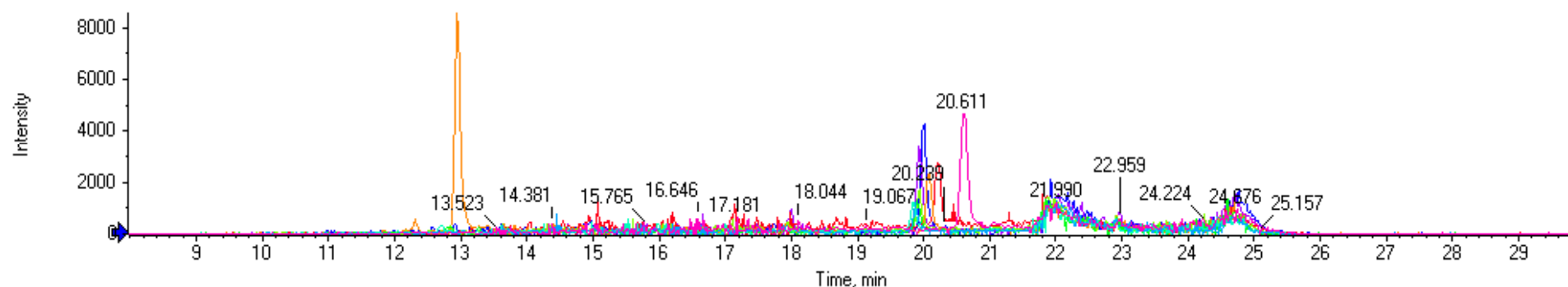
Nanosecond time-of-flight



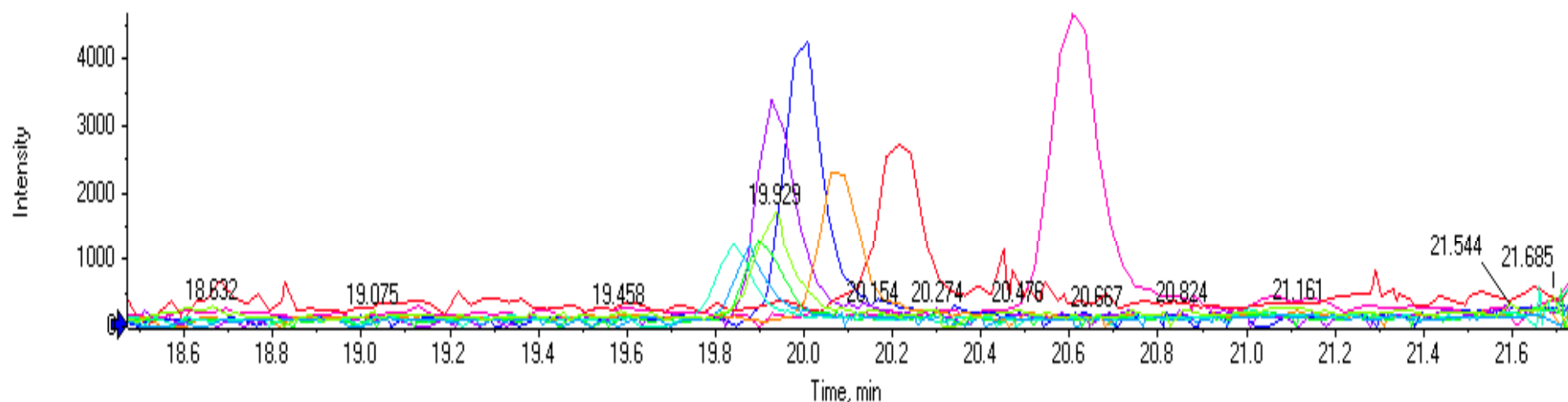


- XIC from 001.wiff (sample 1) - 1, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 002.wiff (sample 1) - 2, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- **XIC from 004.wiff (sample 1) - 4, Experiment 1, +TOFMS (350 - 1250): 709.364 +/- 0.025 Da**
- XIC from 005.wiff (sample 1) - 5, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 006.wiff (sample 1) - 6, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 007.wiff (sample 1) - 7, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 009.wiff (sample 1) - 9, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 010.wiff (sample 1) - 10, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 011.wiff (sample 1) - 11, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da

Samples 1-11

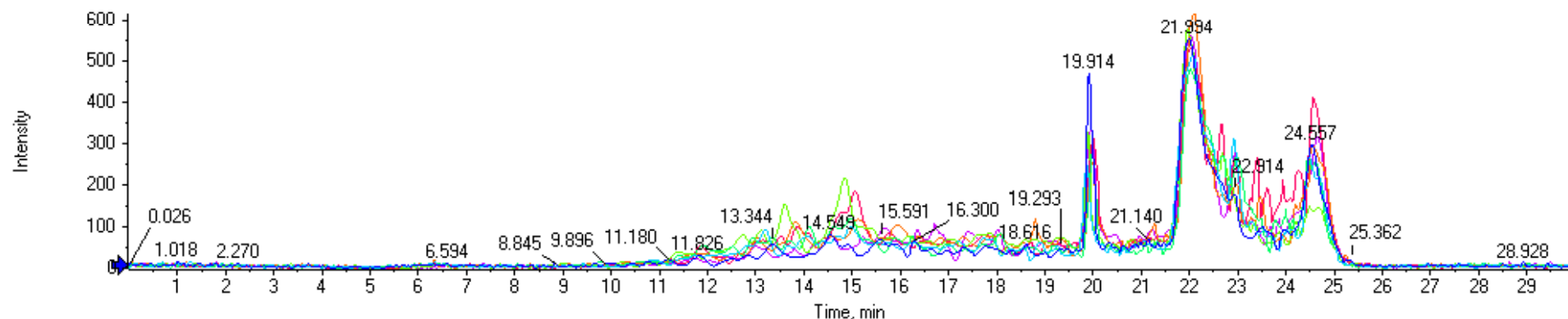


- XIC from 001.wiff (sample 1) - 1, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 002.wiff (sample 1) - 2, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 004.wiff (sample 1) - 4, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 005.wiff (sample 1) - 5, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 006.wiff (sample 1) - 6, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- **XIC from 007.wiff (sample 1) - 7, Experiment 1, +TOFMS (350 - 1250): 709.364 +/- 0.025 Da**
- XIC from 009.wiff (sample 1) - 9, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 010.wiff (sample 1) - 10, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da
- XIC from 011.wiff (sample 1) - 11, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.025 Da

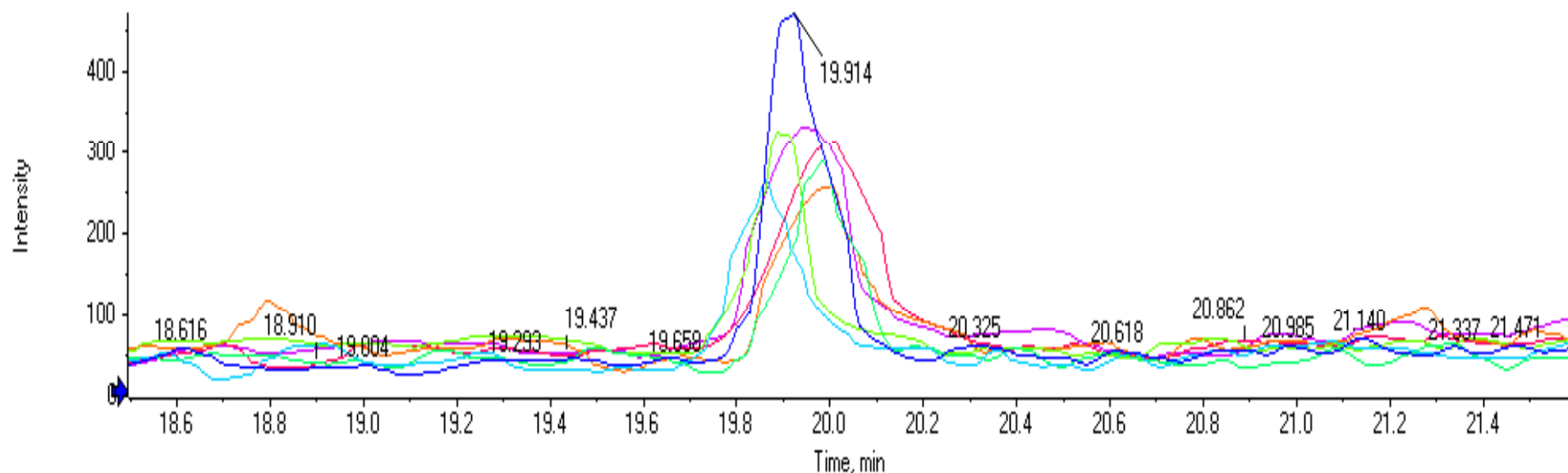


● XIC from 012.wiff (sample 1) - 12, Experiment 1, +TOFMS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 013.wiff (sample 1) - 13, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 014.wiff (sample 1) - 14, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 015.wiff (sample 1) - 15, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 016.wiff (sample 1) - 16, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 017.wiff (sample 1) - 17, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 018.wiff (sample 1) - 18, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed

Samples 12-18

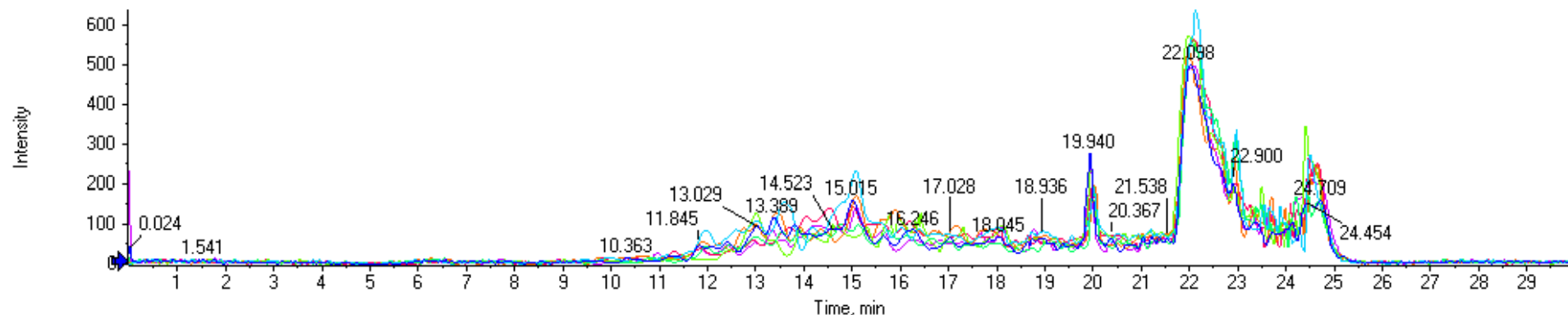


● XIC from 012.wiff (sample 1) - 12, Experiment 1, +TOFMS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 013.wiff (sample 1) - 13, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 014.wiff (sample 1) - 14, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 015.wiff (sample 1) - 15, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 016.wiff (sample 1) - 16, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 017.wiff (sample 1) - 17, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 018.wiff (sample 1) - 18, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed

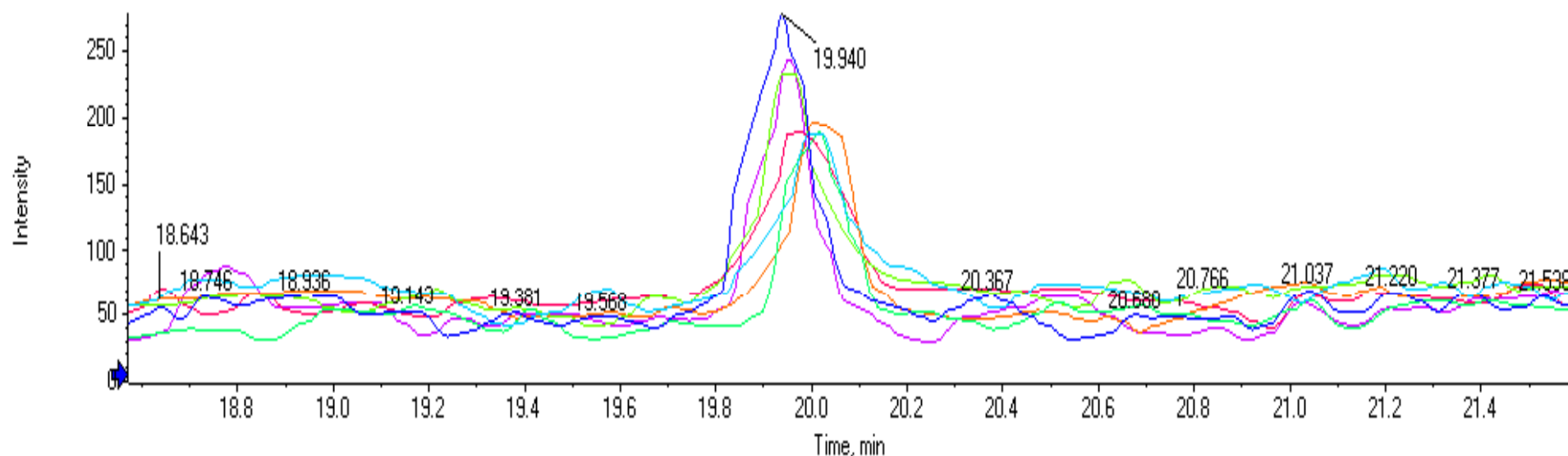


● XIC from 019.wiff (sample 1) - 19, Experiment 1, +TOFMS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 020.wiff (sample 1) - 20, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 021.wiff (sample 1) - 21, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 023.wiff (sample 1) - 23, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 024.wiff (sample 1) - 24, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 025.wiff (sample 1) - 25, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 026.wiff (sample 1) - 26, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed

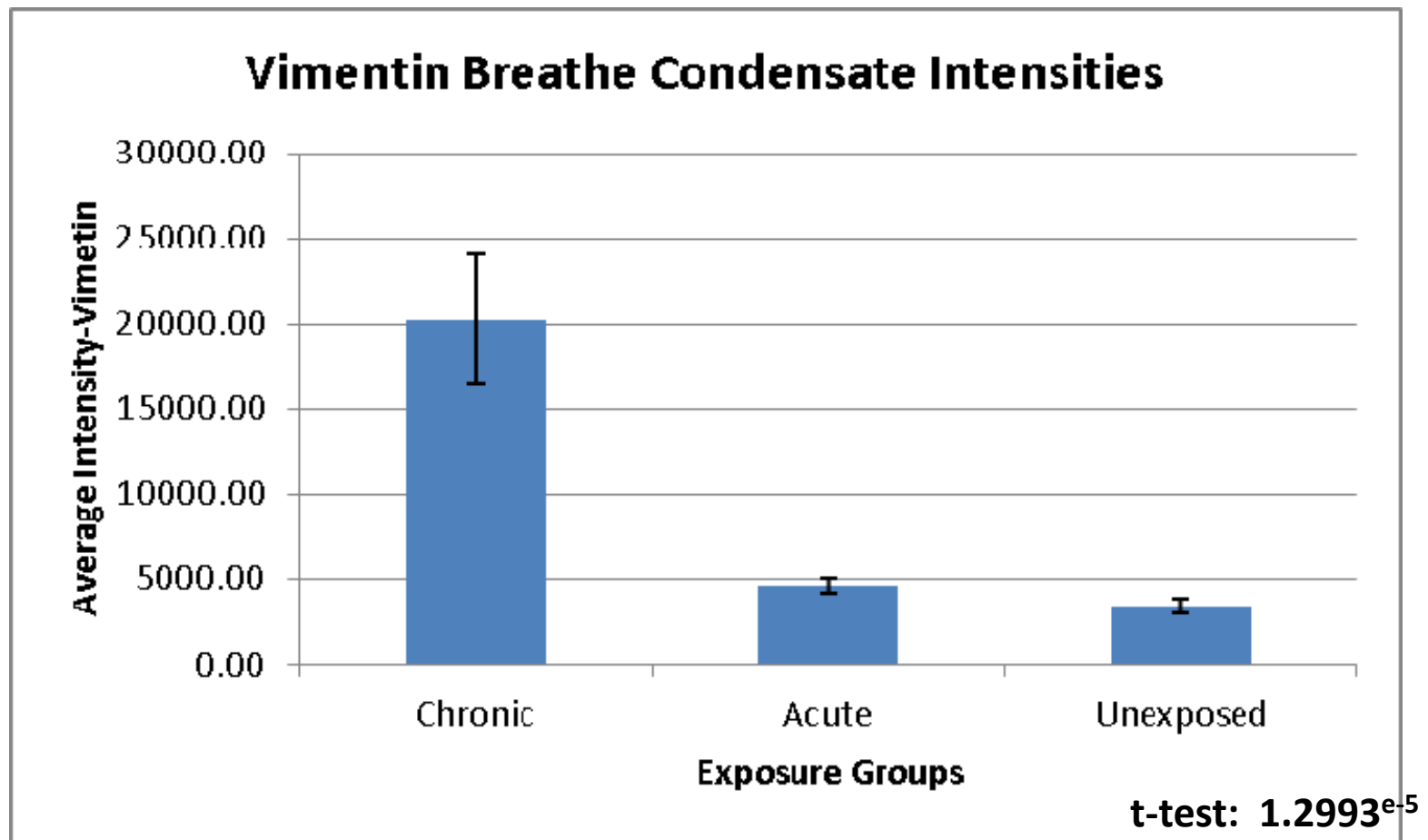
Samples 19-26



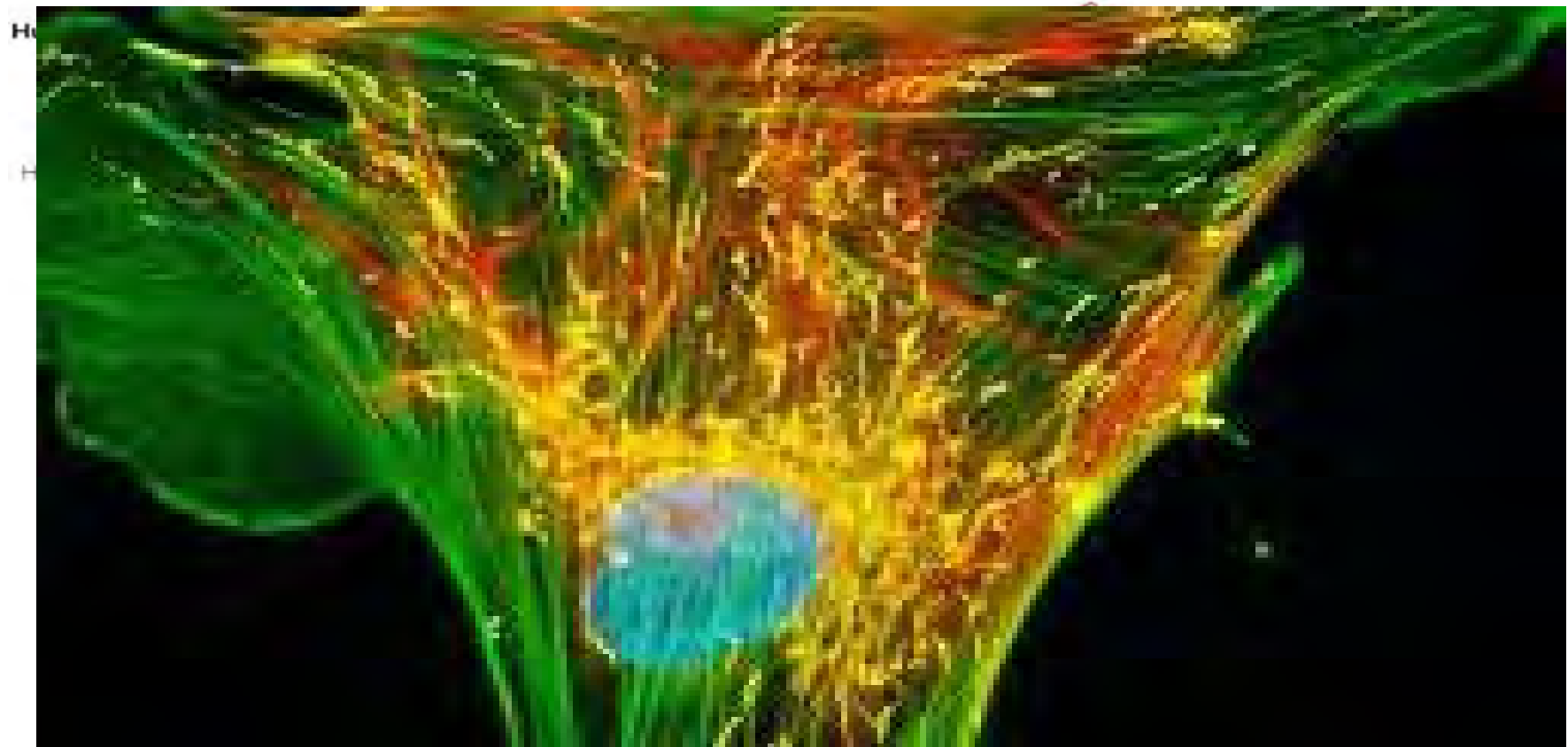
● XIC from 019.wiff (sample 1) - 19, Experiment 1, +TOFMS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 020.wiff (sample 1) - 20, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 021.wiff (sample 1) - 21, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 023.wiff (sample 1) - 23, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 024.wiff (sample 1) - 24, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 025.wiff (sample 1) - 25, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed
 ● XIC from 026.wiff (sample 1) - 26, Experiment 1, +TOF MS (350 - 1250): 709.364 +/- 0.015 Da, Gaussian smoothed



Vimentin levels were significantly higher in people with chronic exposure



What is Vimentin



Vimentin Fragment

LLQDSVDFSLADAINTEFK

- Vimentin fragments are a prognostic marker in synovial fluid of patients with Rheumatoid Arthritis.
- Vimentin fragments have been found in patients with exacerbations of Asthma.
- Vimentin fragments are an “eat me” signal when outside of cells.
- Vimentin fragments spearhead inflammation.

Conclusions

- Exhaled breath condensate is a non invasive method for evaluating lung sensing mechanisms against environmental injury
- The presence of Vimentin in EBC is a marker of lung responses to perturbations in its environment

Conclusions

- Cross disciplinary studies are critically needed to remove the artificial barriers of scientific silos.
- We thank Dr. Sergey Mirov and Dr. Vohra for opening doors and developing platforms where we can collaborate!

Acknowledgements

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