



# BREATH DOSIMETRY: A NON- INVASIVE TOOL TO ASSESS EXPOSURE TO HAZARDS

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## Biological Agent Attack on City of 1,000,000 people

Source: World Health Organization, *Health Aspects of Chemical and Biological Weapons* (Geneva: World Health Organization, 1970), pp. 95-99.

Disease	People at Risk	Estimated # Deaths	Estimated #
<b>Incapacitated</b>			
<b>Anthrax</b>	180,000	95,000	30,000
<b>Brucellosis</b>	1	<b>Take home message: Identifying people who are at greatest risk reduces:</b> 1) the impact of bioterrorism on medical services, 2) the need to stockpile large amounts of drugs/vaccines 3) provides victims with the greatest chance for survival via early treatment.	79,600
<b>Epidemic typhus</b>	1		50,000
<b>Plague</b>	1		36,000
<b>Q fever</b>	1		124,850
<b>Tularemia</b>	180,000	30,000	95,000
<b>Venezuelan equine encephalitis</b>	60,000	200	19,800

The WHO model assumes a city of 1,000,000 people in a developed country, and makes assumptions regarding the population distribution around a high density urban core that may no longer be appropriate. The model also makes certain assumptions about the agent (50 kilograms of dried powder containing  $6 \times 10^{15}$  organisms disseminated in a line 2 kilometers long at a right angle to the wind direction. The model nominally illustrates dissemination from an aircraft, but none of the calculations appears to depend on the type of the delivery vehicle involved. As an example, the model assumes that the Venezuelan equine encephalitis will survive for only about 5-7 minutes, during which time it will travel about 1 kilometer. About 60,000 people will be exposed to the agent. About 20,000 people will become incapacitated, including 200 who will die. In contrast, anthrax will survive for more than two hours and will travel for more than 20 kilometers. At least 180,000 people will be exposed to the agent, including 30,000 who will become incapacitated and 95,000 who will die.

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## Non-Invasive Detection of Pulmonary Pathogens

### Critical Challenge:

To develop a rapid diagnostic method to assess infection by aerosol agents.

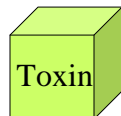
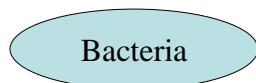
### Approach:

- Focus on site of infection: **Respiratory system**
- Select non-invasive detection method to reduce risk to patient and operator and improve speed and convenience of analysis: **Breath Analysis**
- Select analysis technique which is rapid, sensitive and portable: **mass spectrometry**

**CRITICAL ISSUE: WHAT SHOULD WE MEASURE?**

## Concept of Operations

### AIRBORNE MICROBES



Virus

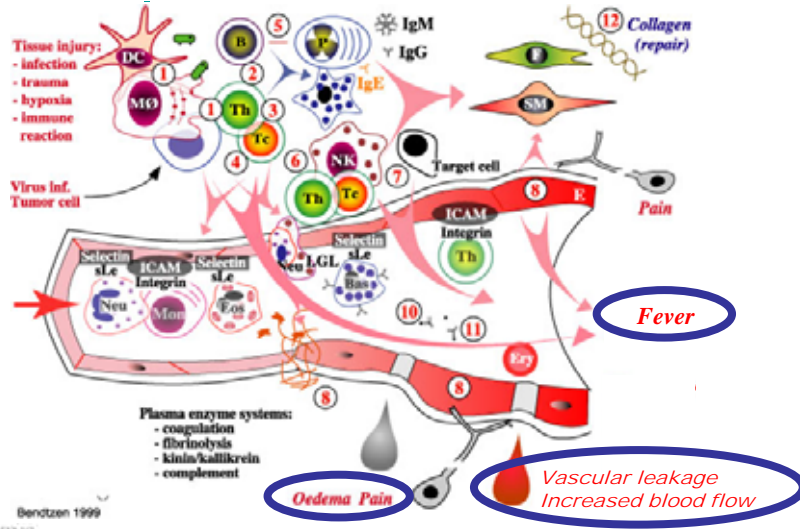
1. Microorganism enters lungs and triggers an inflammatory response.



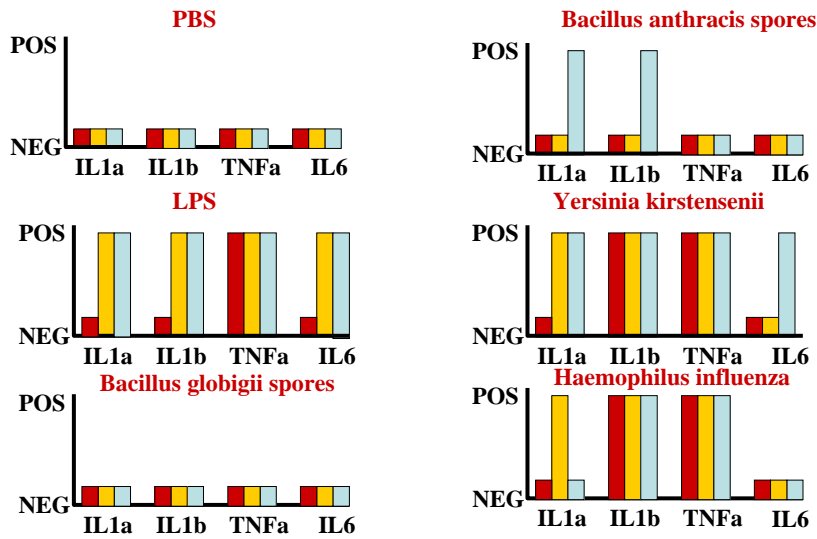
**Discriminate:**  
**Infected vs. Non-infected?**  
**Bacterial or Viral?**  
**Which microbe?**



# The Inflammatory Response



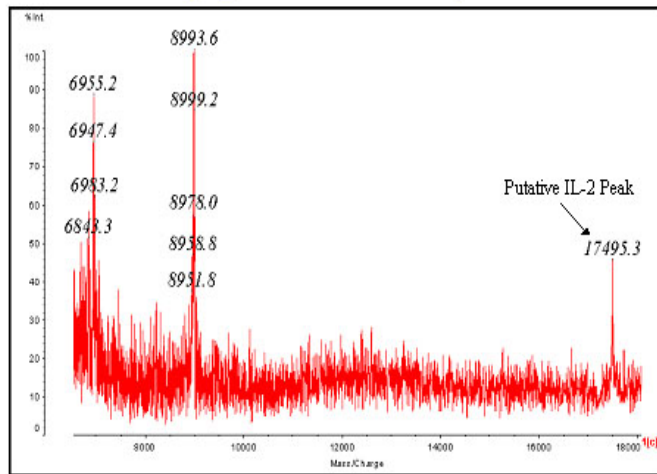
## Cytokines Expression Pattern Changes with Agent



## MALDI TOF mass spectrometric detection of cytokines in swine breath in response to SEB exposure PRIOR TO SYMPTOMS

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Detection as early as 1 hour following 50 ug/kg dose Staphylococcus enterotoxin B



ELISA assays confirmed the presence of IL-2 and the absence of other cytokines in exhaled breath condensates

Further confirmation of peak identification underway using protein sequencing by MALDI-TOF.

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## Baseline Values of Cytokines in EBC are undetectable in healthy animals

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Arranged to purchase breath samples from a commercial pig farm with a diverse genetic background of pigs.

Obtained random samples of EBC from masked piglets weighing between 3.4 kg to 9.5 kg.

Obtained health data on pigs for period of one week post sampling.

Using ELISA assays as gold standard surveyed samples for early and mid cytokines,  $\text{TNF}\alpha$  and IL-6.

There is no induction of these cytokines in healthy animals



Farmhand performs "in sty" collections



Piglet is sleeping during collection

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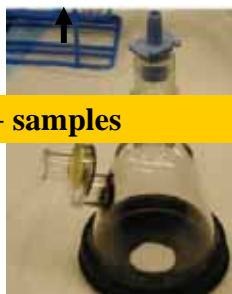
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### Optimizing Sampling Conditions for EBC in Noninfected Humans Volunteers

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**67% + samples**

versus

**33%+ samples**



APL Modified mask

ECO SCREEN

- No significant adverse events
- Defined Minimum collection time for ED subjects 15 minutes
- Of all subjects with detectable protein - no cytokines detected
- Mass spectrometric patterns of healthy people more complex than that observed in healthy piglets

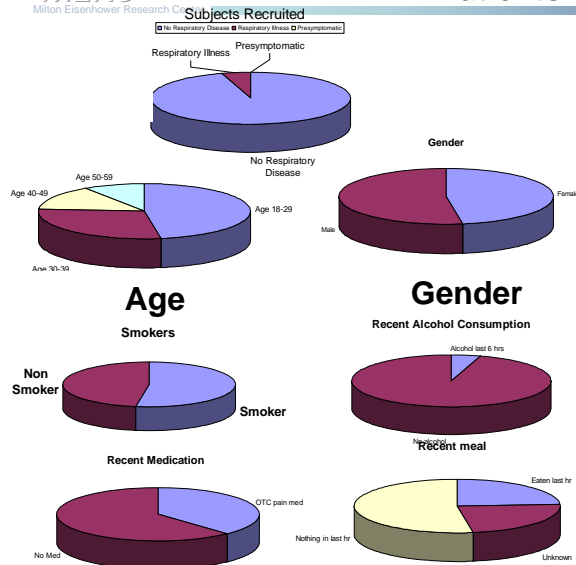
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## Early Recruitment Biased Toward Non Respiratory Patients

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**Total of 72 subjects recruited**

**No pre-symptomatic patients recruited**

**No contribution observed due to recent meal**

**Significant effect on sample content from smoking**

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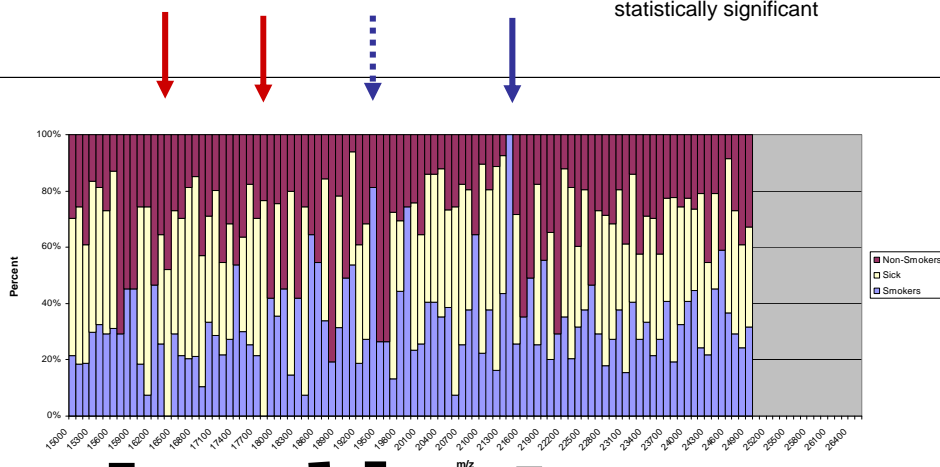
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## Peak Prevalence as Percentage of All Observations

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For mass range 15-30 kDa

\*N.B. Data from ill patients not statistically significant



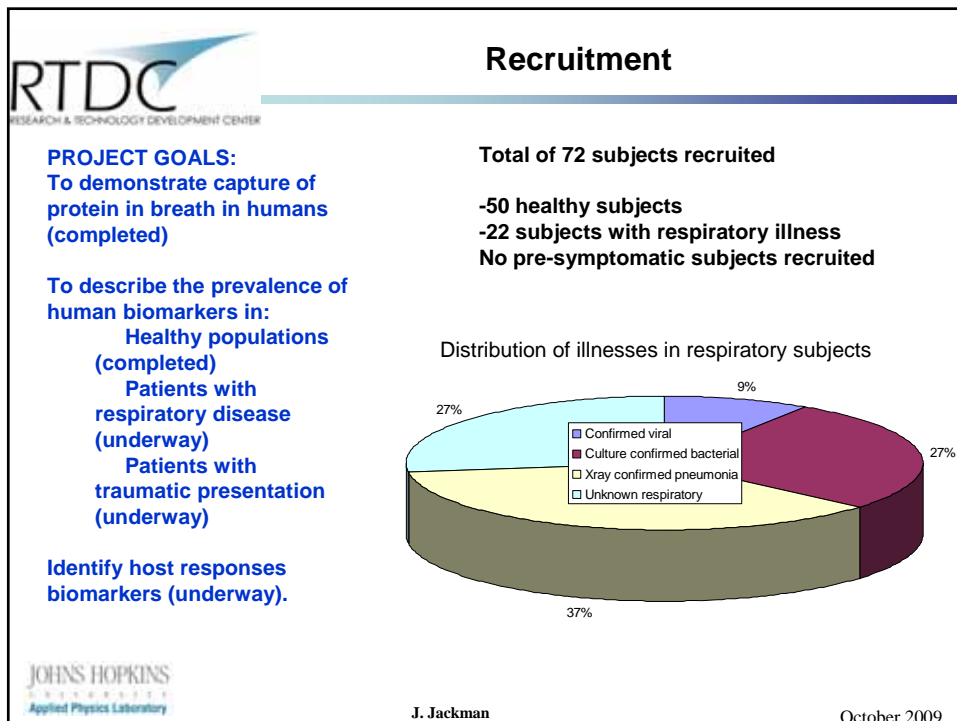
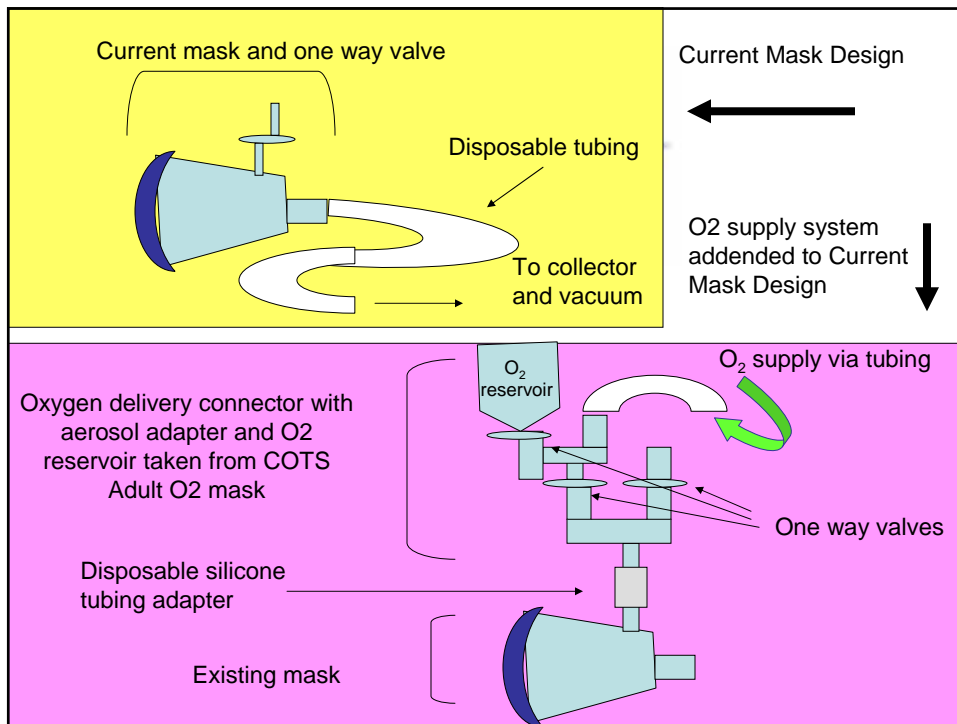
**Candidate peaks in smokers (blue), non smokers (red) and absence of peaks in ill patients (black regions)\***

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APL Proprietary

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## ED Subjects

### Sample Characteristics

#### Subjects presenting with respiratory illness were sampled

- All respiratory patients gave two breath samples at time of initial visit
- 2 respiratory patient returned within two weeks and gave two more breath samples

#### Subjects presenting in ED with other illnesses (non-respiratory complaint/trauma) were sampled

- All subjects gave two breath samples at time of initial visit
- 3 subjects returned to give an additional sample.

#### Assayed by multiplex ELISA for 19 cytokines

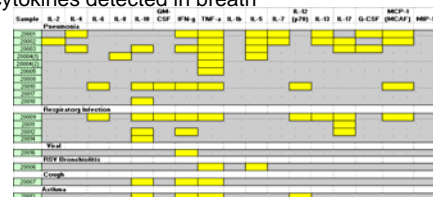
(sensitivity to 0.2 pg for select cytokines) & for mass spectral protein profiles

Samples assayed "neat" without sample concentration or sample preparation prior to addition to assay.

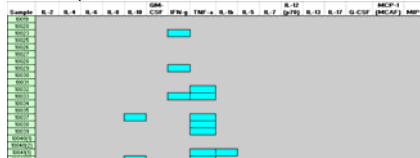
- MALDI samples washed "on probe" during application of matrix and sample

### Cytokines detected in human EBC:

Respiratory challenged subjects 16 out of 17 cytokines detected in breath

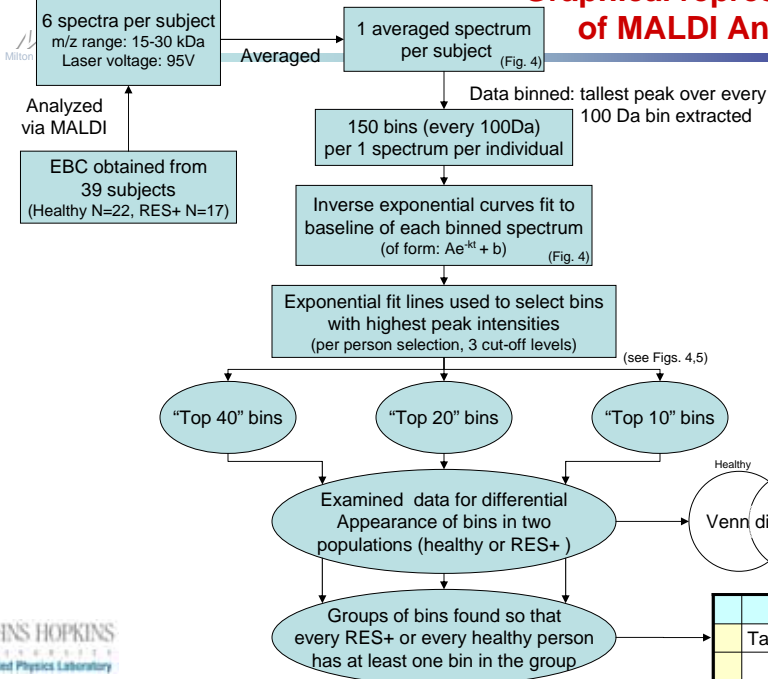


Healthy subjects: detected four cytokines at low (at or below LOQ)



12 of 17 cytokines only found in respiratory pts.

## Graphical representation of MALDI Analysis

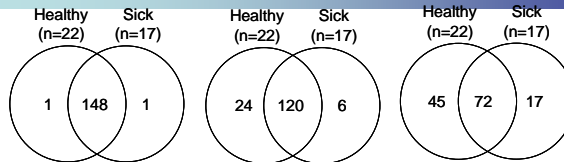


## Preliminary MALDI Analysis and Data Reduction of Mass Peaks

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Unique bins

100Da bins from  
15.0 to 29.9 kDa  
150 total bins



~50  
million  
protein  
sized  
peaks

Manual Data Reduction

Representative bins

Peaks picked per person	"Top 40 " Least exclusive (1674 total peaks)	"Top 20 " Middle exclusive (829 total peaks)	"Top 10 " Most exclusive (392 total peaks)
All healthy (% of subjects) N=22	1 unique bin (45.5%)	8 of 24 unique bins (95.5%)	9 of 45 unique bins (90.9%)
All sick (RES+) (% of subjects) N=17 people	1 unique bin (23.5%)	5 of 6 unique bins (52.9%)	8 of 17 unique bins (94.1%)

+16 bins not present in anyone

### Conclusions:

- Unique bins were found that were present only in healthy or only in RES+ individuals
- If the "Top 10" bins per individual are examined, 90.9% of all healthy people have at least 1 bin of a group of 9 and 94.1% of RES+ individuals have at least one bin out of a group of 8 bins

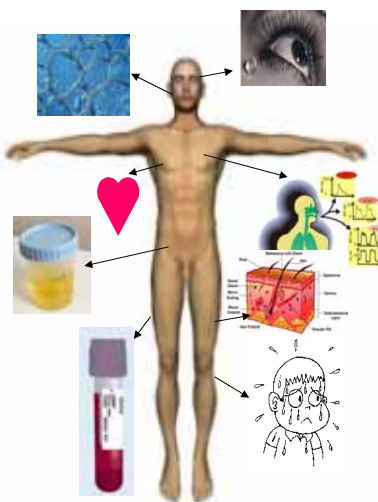
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## Exploiting the signals produced by humans for better diagnostics

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What diagnostic compartments in addition to blood can be readily exploited for diagnostics?

**Breath:** measures lung function, carries waste from the body, indicates oxygenation and oxidative stress

**Saliva:** used for detection of small metabolites and drugs of abuse

**Sweat:** used as inexpensive diagnostic for CF

**Urine:** primary carry of waste, used to monitor metabolic balance, proteinuria and specific gravity are current diagnostics

**Tears:** has not been widely exploited likely due to sample size and excessive salinity

**Blood:** carries nutrients to cells, transport waste, commonly analyzed for small molecules, whole cells, hormones and inflammatory markers.

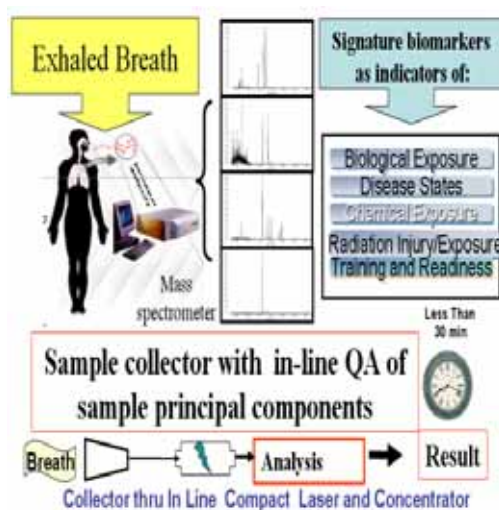
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## SUMMARY



### Advantages of breath as a diagnostic:

- Non-invasive
- Low impact
- Rapid Turnaround
- Easier sample to analyze than blood

-Demonstrated collection of cytokines in both humans and swine breath.

-Detected by ELISA or direct sequencing following markers in breath: oxytocin (trust), CRP, Immunoglobulins, T cell receptor genes

-Demonstrated appearance of "wellness" markers in both humans and swine breath.

-Demonstrated pre-symptomatic detection of disease in swine (anticipate humans to show same response).

-Demonstrated the presence of markers of chemical exposure to days after exposure.

## Acknowledgements

### • Sampling:

- Carousel Angus Farm (Taneytown, MD)
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