

Johns Hopkins Engineering

Immunoengineering

Immunoengineering—Allergy and Autoimmunity

Diagnostics



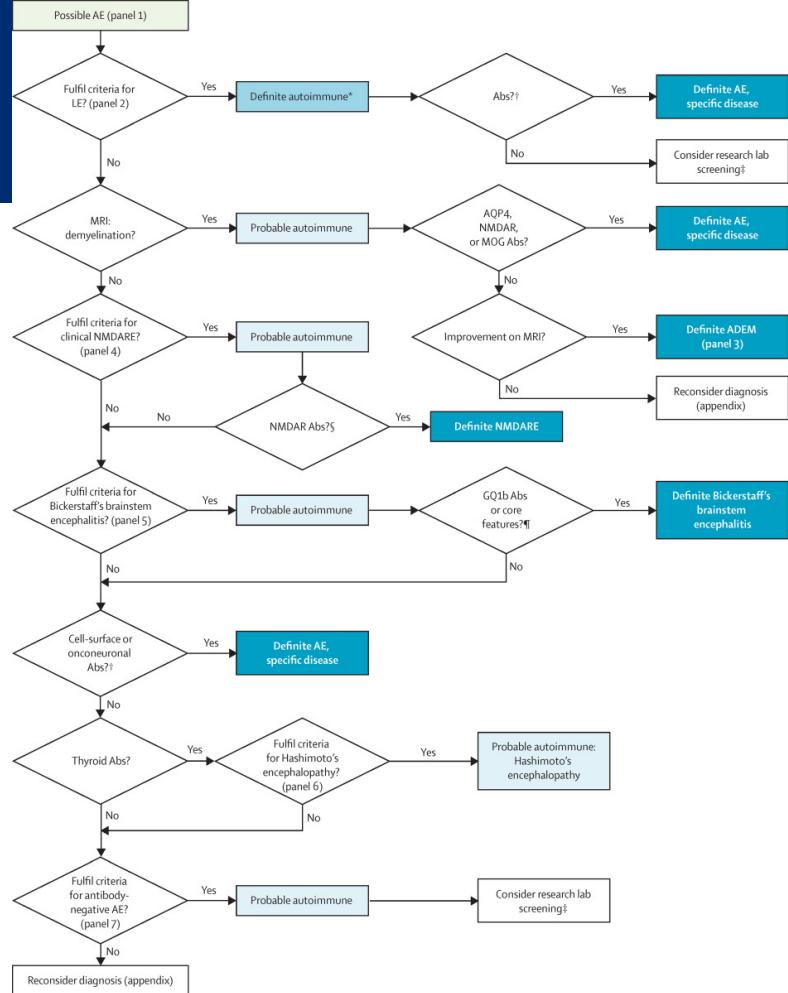
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of ENGINEERING

Considerations for Diagnostic Development

- Encephalitis Example
 - Integration
- Asthma Example
 - Sensitivity and specificity
 - Validation with gold standard
- Food Allergy Example
 - Multiplexing
 - Sample amount and processing time
 - Cost and complexity
 - Sample source and readout

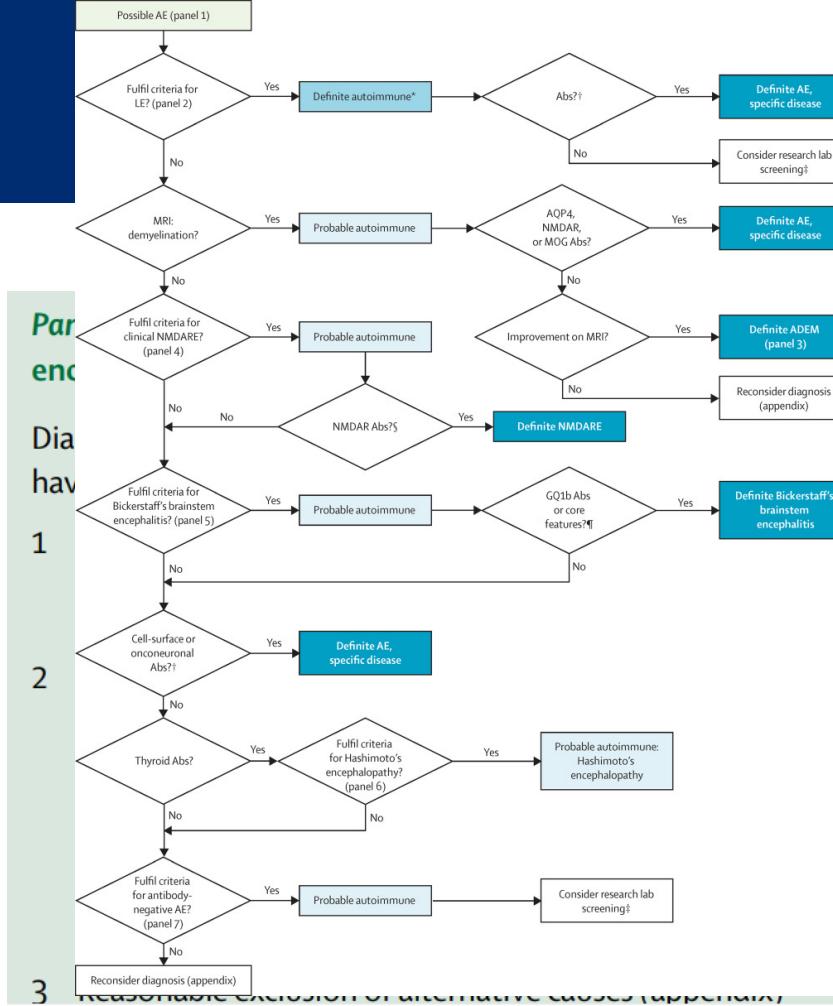
Encephalitis Example

- Where will your test fall in the decision tree?
- Even within a disease there are differential diagnoses
 - Especially true for autoimmune disorders where not much is known



Encephalitis Example

- Where will your test fall in the decision tree?
- Even within a disease there are differential diagnoses
 - Especially true for autoimmune disorders where not much is known
- Physicians need to judge based on symptoms
 - Because of expense and time it takes for results of tests



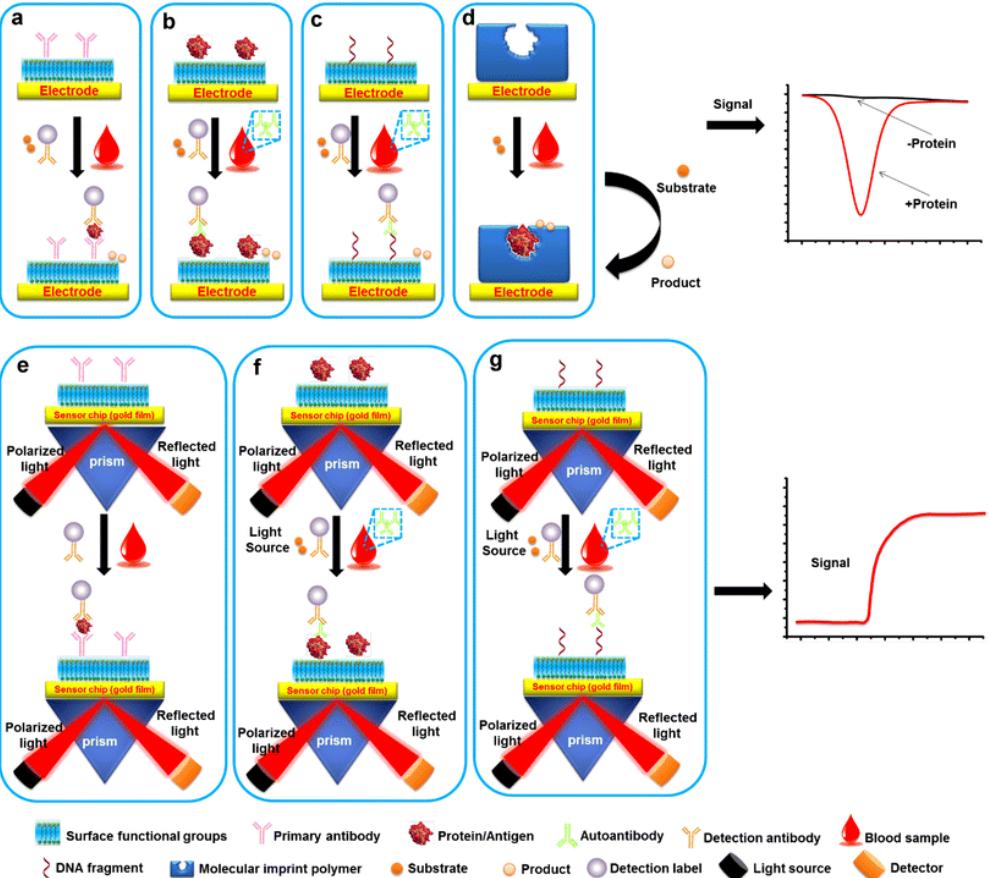
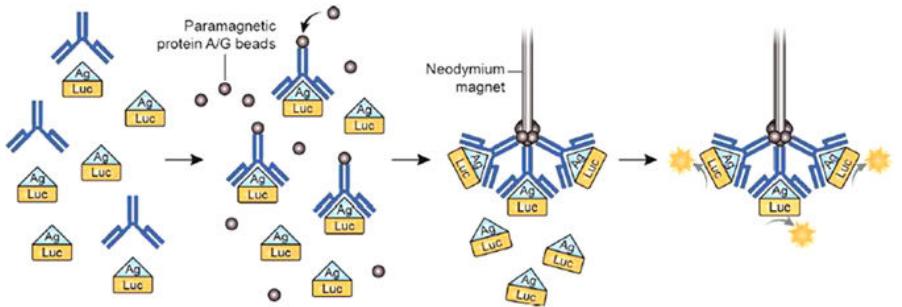
Encephalitis Example

- Importance of recognizing previous diagnostic approaches
- Will your test compete or integrate previous approaches?
- If new then linking physiology to marker

	Syndrome	Diagnostic assay	Frequency of cancer	Main type of cancer
Antibodies against intracellular antigens				
Hu (ANNA1) ^{1*}	Limbic encephalitis	Western blot	>95%	Small-cell lung carcinoma
Ma2 ²	Limbic encephalitis†	Western blot	>95%	Testicular seminoma
GAD ¹⁰	Limbic encephalitis‡	Radioimmunoassay	25%§	Thymoma, small-cell lung carcinoma
Antibodies against synaptic receptors				
NMDA receptor ¹¹	Anti-NMDA receptor encephalitis	Cell-based assay	Varies with age and sex	Ovarian teratoma¶
AMPA receptor ¹²	Limbic encephalitis	Cell-based assay	65%	Thymoma, small-cell lung carcinoma
GABA _A receptor ¹³	Limbic encephalitis	Cell-based assay	50%	Small-cell lung carcinoma
GABA _A receptor ¹⁴	Encephalitis	Cell-based assay	<5%	Thymoma
mGluR5 ¹⁵	Encephalitis	Cell-based assay	70%	Hodgkin's lymphoma
Dopamine 2 receptor ¹⁶	Basal ganglia encephalitis	Cell-based assay	0%	..
Antibodies against ion channels and other cell-surface proteins				
LGI1 ¹⁷	Limbic encephalitis	Cell-based assay	5–10%	Thymoma
CASPR2 ¹⁸	Morvan's syndrome or limbic encephalitis	Cell-based assay	20–50%	Thymoma**
DPPX ¹⁹	Encephalitis††	Cell-based assay	<10%	Lymphoma
MOG ²⁰ ‡‡	Acute disseminated encephalomyelitis	Cell-based assay	0%	..
Aquaporin 4 ²¹ ‡‡	Encephalitis	Cell-based assay	0%	..
GQ1b ²²	Bickerstaff's brainstem encephalitis	ELISA	0%	..

Encephalitis Example

- Autoimmunity is frequently characterized by autoantibodies
- Development focused on new techniques to identify autoantibodies



Asthma Example

- Difficulty in patient compliance with children and elderly
- Misdiagnosis with subpopulations
 - e.g. obese
- Do not characterize disease severity

Questionnaire

- Family history
- Symptoms
- Frequency of exacerbations
- Environmental conditions at work
- Allergies?

Spirometry

- Spirometry measures the max capacity and flow rate of a patient's lungs in order to determine if their airways are constricted.

- FVC • FEV1% predicted • PEF
- FEV • Reversibility • TLC

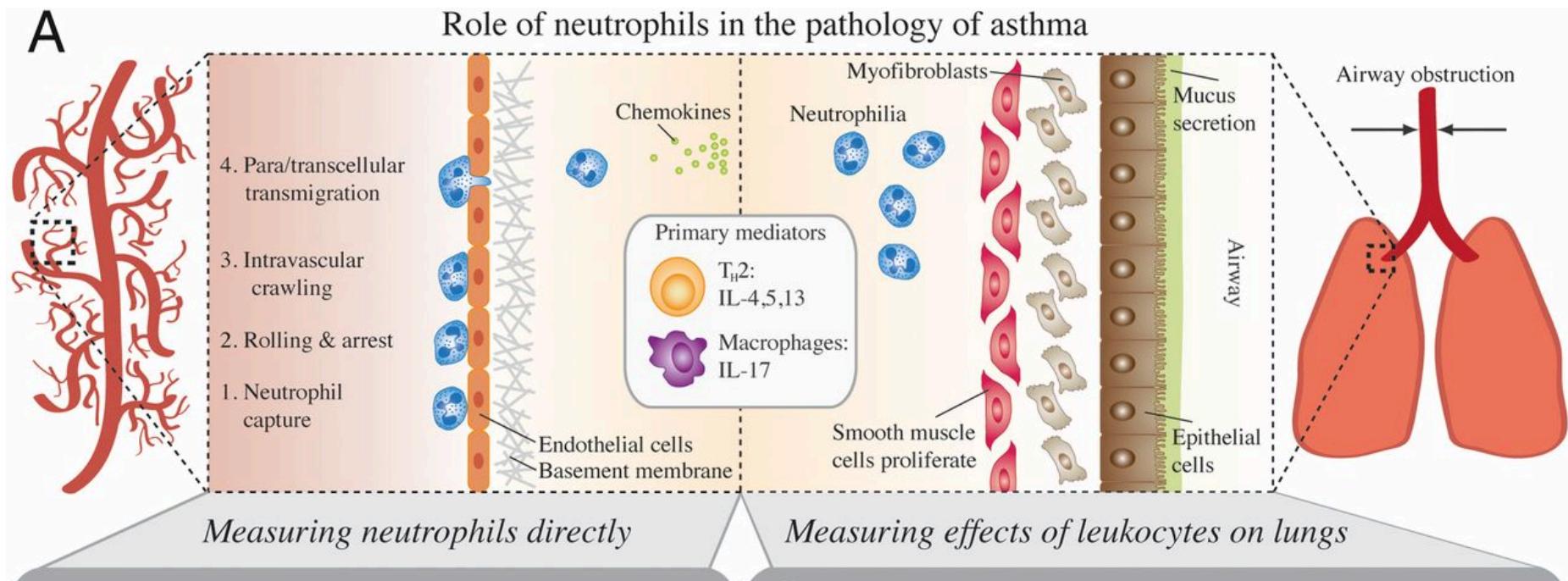
Bronchial challenge

- Physicians may attempt to trigger asthma by challenging the lungs with:
- | | |
|-------------|----------------|
| • Allergens | • Methacholine |
| • Exercise | |

Fractional exhaled nitric oxide (FeNO)

- Measure nitric oxide concentration in patient's breath
- Concentration changes with presence of inflammatory cells
- Higher FeNO measurements indicates possible asthma diagnosis

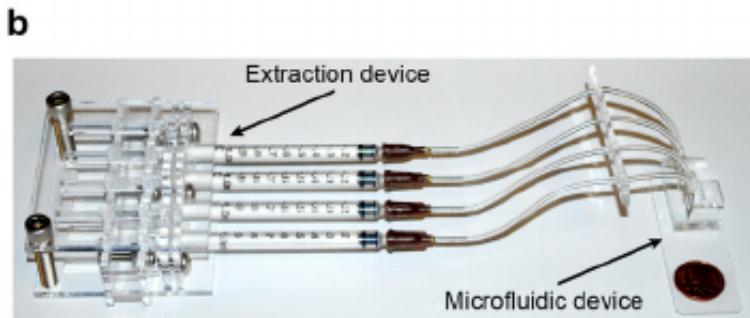
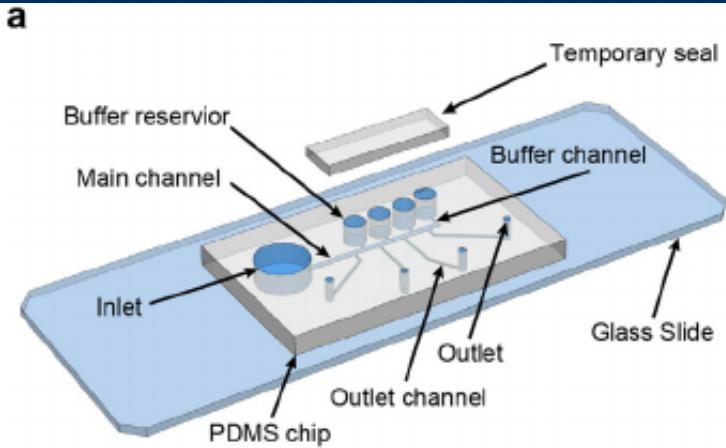
Asthma Example



Sackmann, Eric Karl-Heinz, et al. "Characterizing asthma from a drop of blood using neutrophil chemotaxis." *Proceedings of the National Academy of Sciences* 111.16 (2014): 5813-5818.

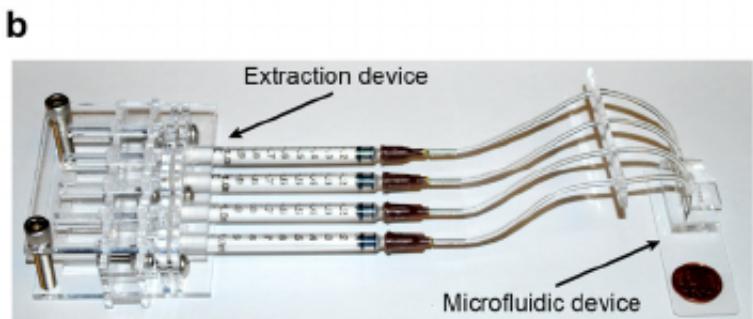
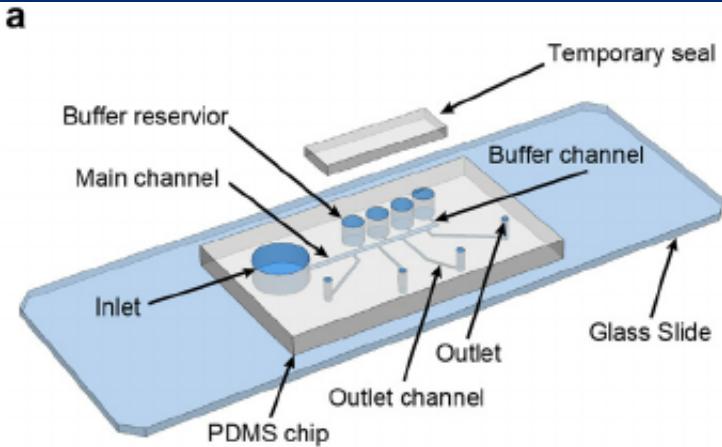
A tangent - Microfluidics

- Decrease necessary reagent and sample input
- Increase sensitivity due to low volume
- Integration of multiple processing steps
- Decreased processing time



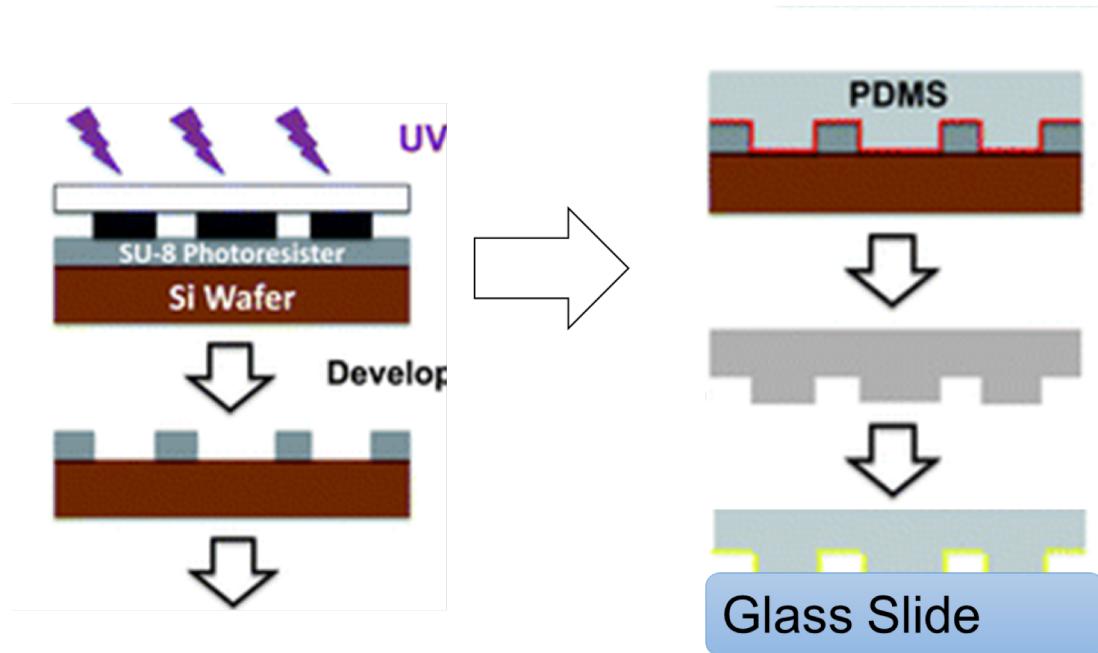
A tangent - Microfluidics

- Require sample preparation
- Necessity for external syringe pumps
- Reliability
- Manufacturability



A tangent - Microfluidics

- Traditionally produced by lithographic techniques
- PDMS bonded to glass coverslip

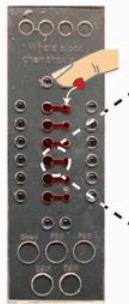


Asthma Example

- Purify Neutrophils
- Add chemokine containing gel
- Neutrophil chemotactic ability
 - Migration speed
 - Chemotactic index
 - Chemotactic velocity
- Automate tracking analysis

B Microfluidic chip diagnostic measurement

1. Purify neutrophils from whole blood in “base”

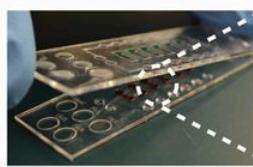


Neutrophil capture on P-selectin substrate

Purify neutrophils with laminar flow wash

flow

2. Place “lid” onto “base” to initiate the assay



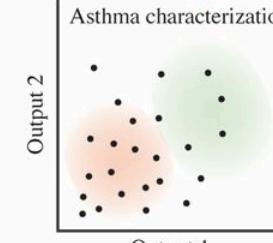
Source

Sink

3. Analysis



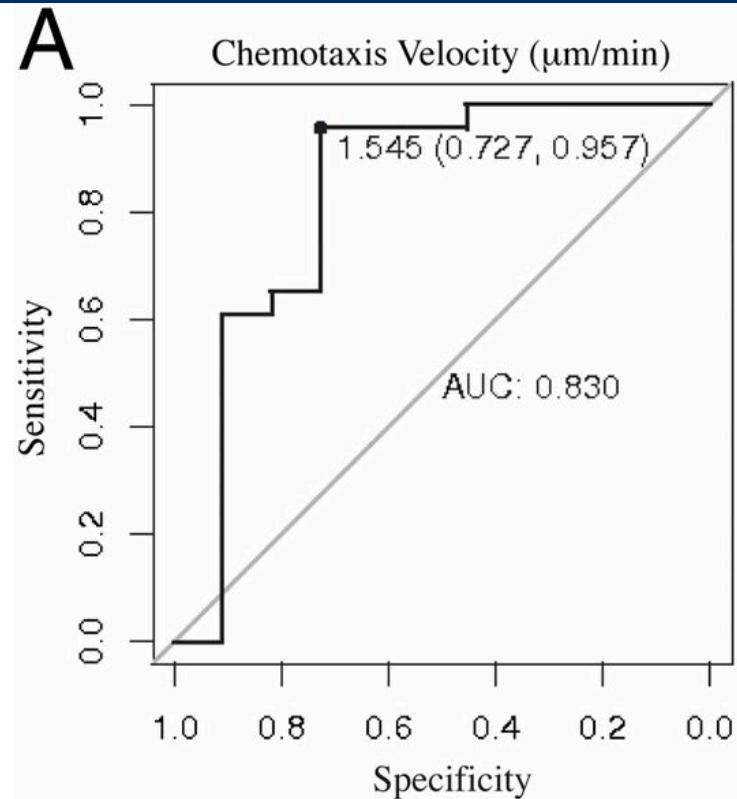
Analyze neutrophil chemotaxis tracks and generate outputs:
1) absolute speed
2) chemotactic index
3) chemotaxis velocity



Output 1

Asthma Example

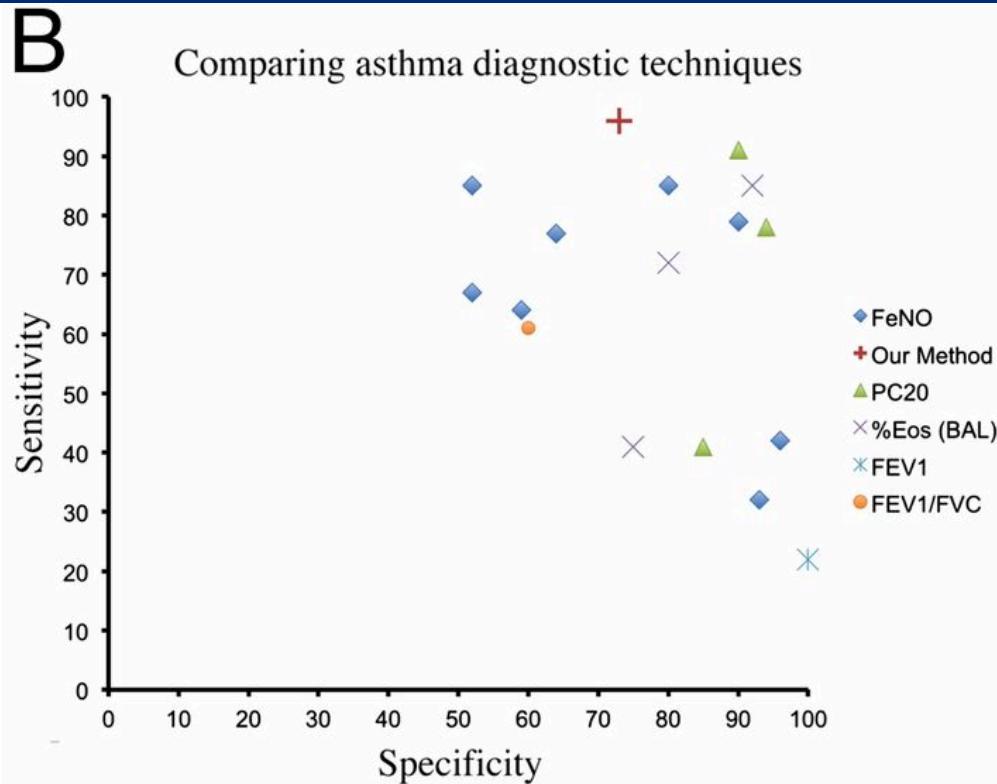
- Determination of optimal specificity and sensitivity cut-off for biomarker
- Sensitivity
- Specificity



Sackmann, Eric Karl-Heinz, et al. "Characterizing asthma from a drop of blood using neutrophil chemotaxis." *Proceedings of the National Academy of Sciences* 111.16 (2014): 5813-5818.

Asthma Example

- Comparison to other Gold standard assays

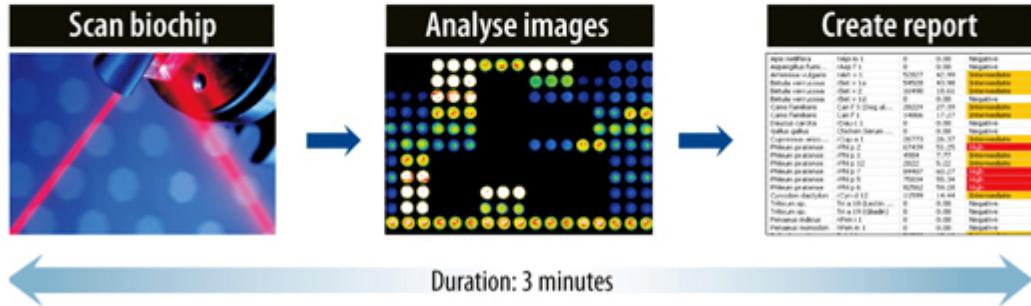
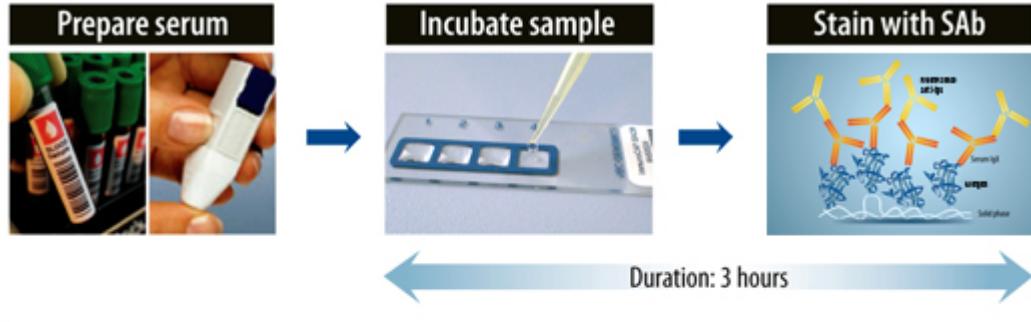


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Allergy Example

- Skin prick tests not necessarily predictive
 - Different immune system
 - Not representative antigens used
 - Incompatibility with skin disease
- Serum IgE levels not always correlated with disease severity
 - IgE levels post immunotherapy
- Low throughput and inability to test for many antigens at once

Allergy Diagnostics



Allergy Diagnostics

- Miniaturizing the antibody assay
- Current IgE require large patient samples
- Solution = Nanostructure Sensors

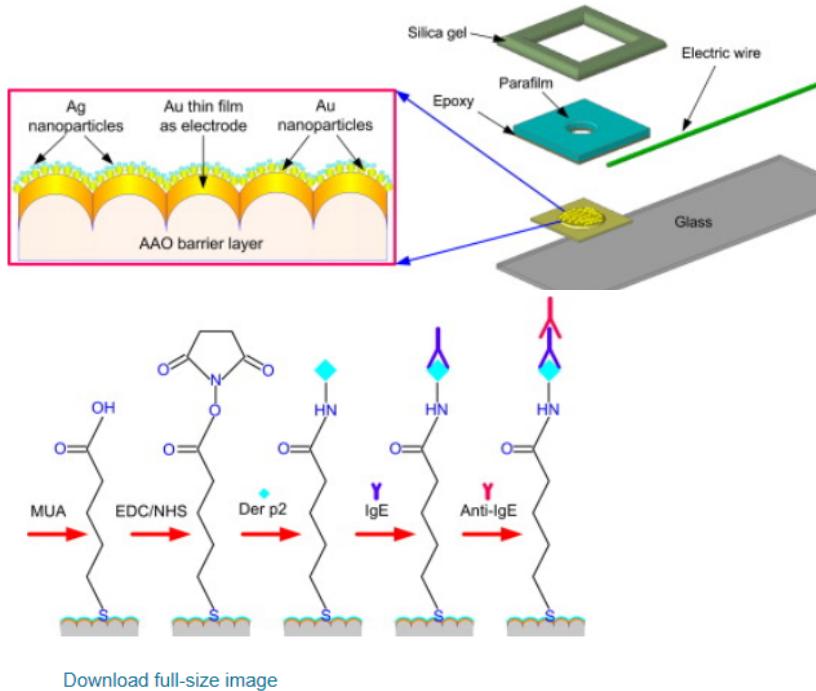
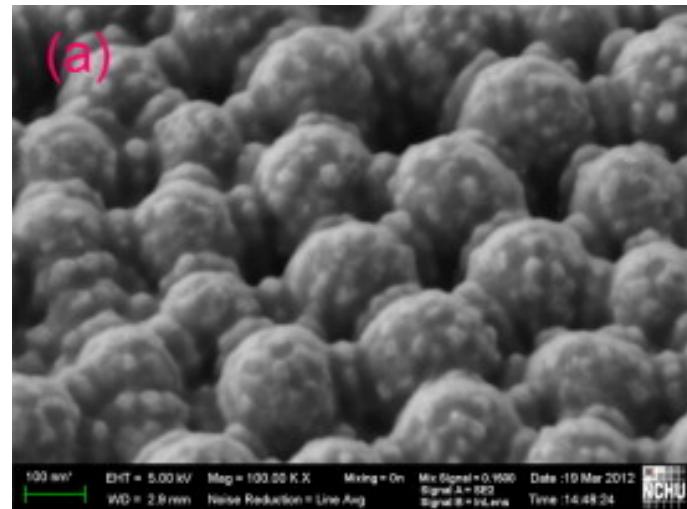


Fig. 2. Immobilization of IgE.

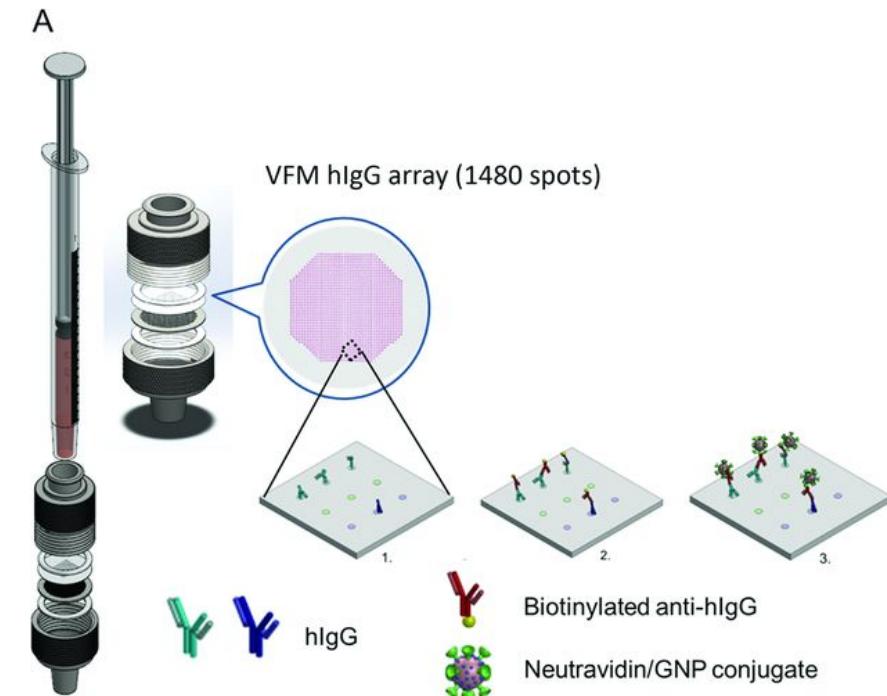
Allergy Diagnostics

- Requires only 25 μL of serum
- <1 minutes of detection time
- Limited current multiplexing
- Manufacturing scalability



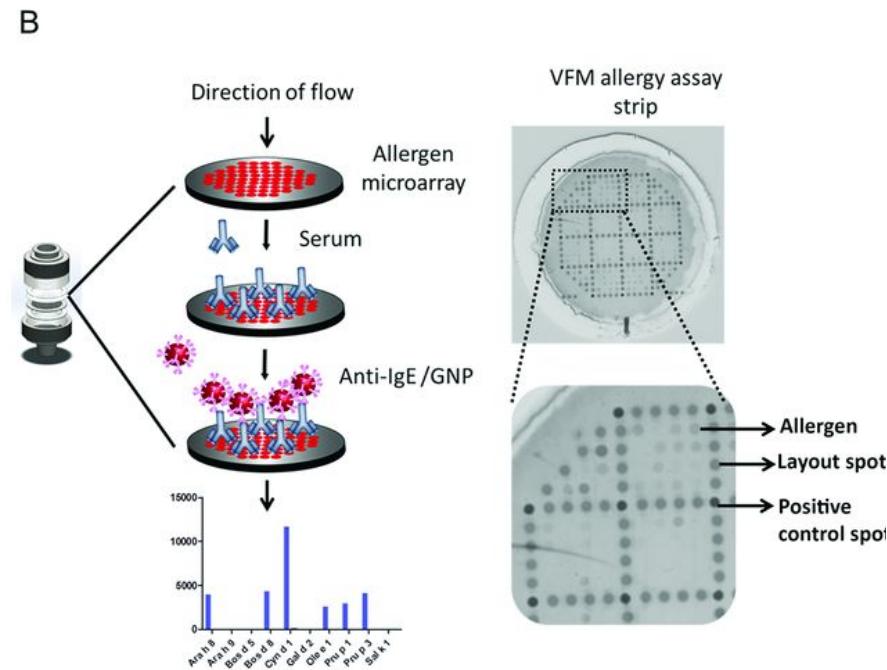
Allergy Diagnostics

- Simplifying the assay
- Current diagnostics require substantial infrastructure – techs/equipment
- Solution = Paper microfluidics array



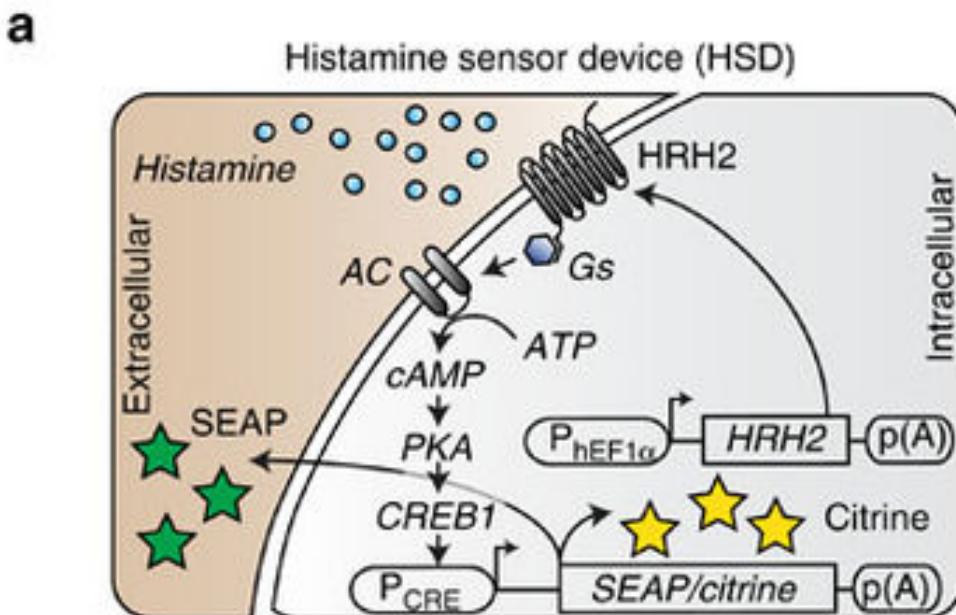
Allergy Diagnostics

- Multiplexing & sample processing easy
- Assay time < 10 minutes
- Insufficient detection and correlation with gold standard



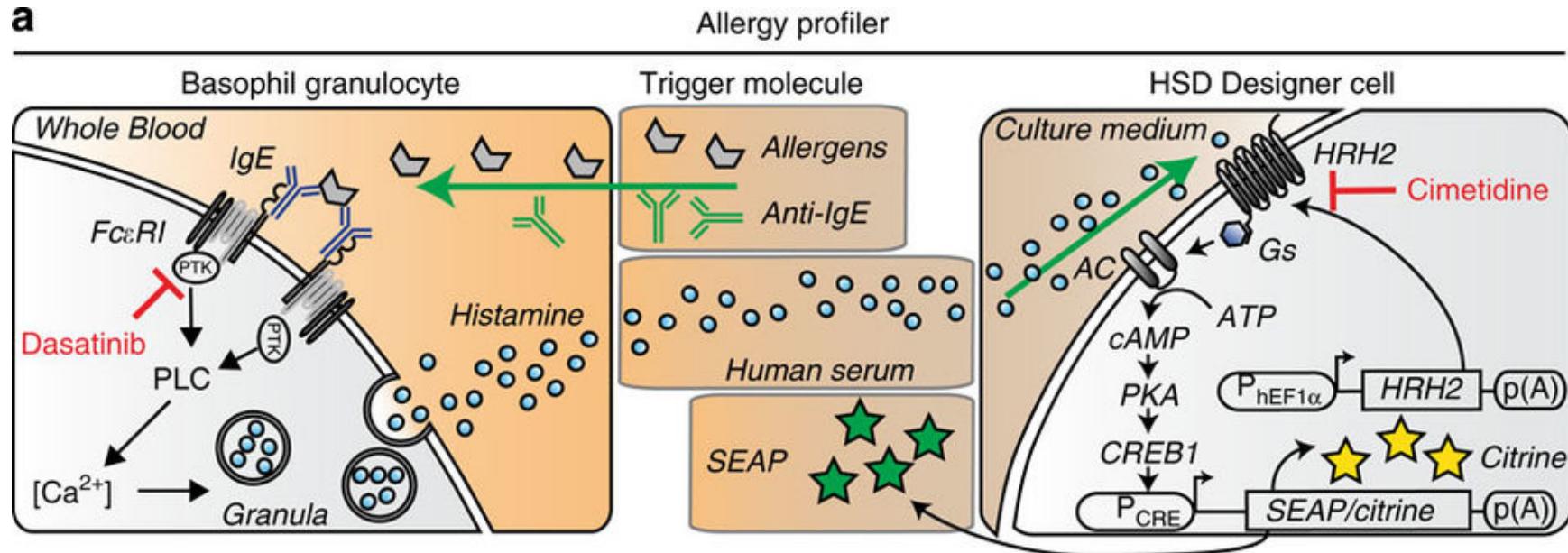
Allergy Diagnostics

- Allergic Reaction in a tube
- Current histamine release tests require large volumes or modification of histamine
- Solution = Synthetic Biology



Synthetic Biology Solution to Allergy Diagnostics

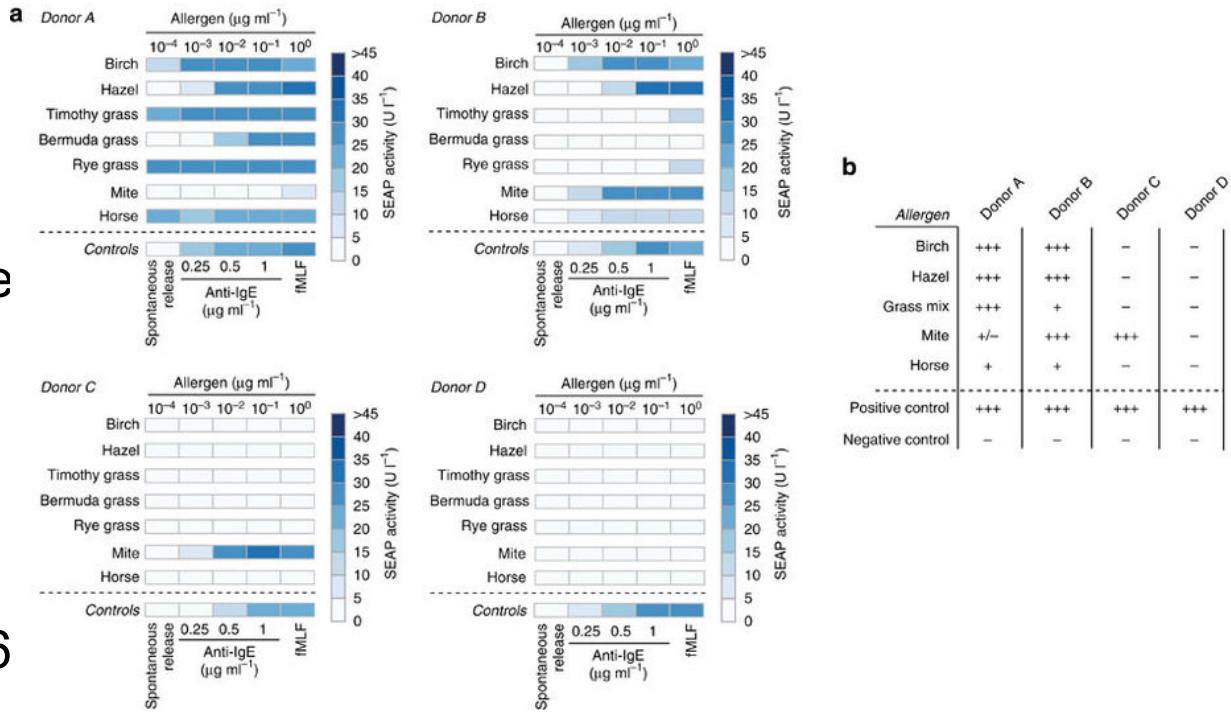
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Synthetic Biology Solution to Allergy Diagnostics

■ Pros

- High throughput
- Sensitivity
- Correlates with skin test
- Ability to test multiple concentrations



■ Cons

- Stability of cell line
- Expense of process and cell handling
- Length of assay (~36 hours)

Not just to test patients but also food



Gluten Sensor

The world's first portable gluten tester



Peanut Sensor

Coming Soon. Reserve yours today.





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