Dialysis Like Therapeutics
Continuous blood sensing, scrubbing, and therapy

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Proposer's Day

February 23, 2011

Program objective

Dialysis Like Therapeutics will create a portable device that continuously senses, scrubs and closed-loop manipulates the entire blood volume

Component Technologies:

- Non-fouling, continuous sensors for complex biologic fluids
- High-flow microfluidic structures that do not require use of anticoagulation
- Intrinsic separation technologies that do not require pathogen specific molecular labels or binding chemistries
- Predictive modeling and control with sufficient fidelity to enable agile and adaptive closed-loop therapy

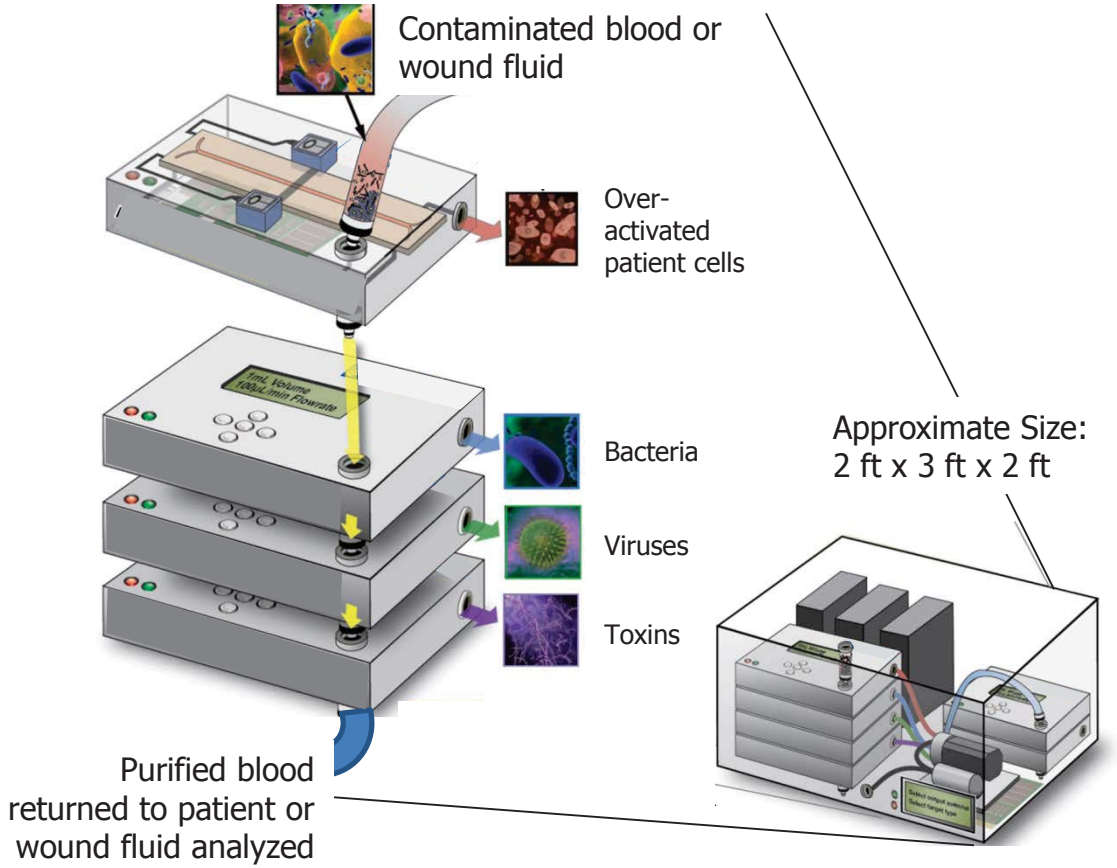


Image adapted with permission from Lawrence Livermore National Laboratory

Program impact

Platform device will revolutionize multiple areas of medicine:

- **Sepsis**
- Wound Infection
- CBRN Defense
- Trauma
- Regenerative Medicine
- Autoimmune
- Cancer
- Diabetes
- Cardiovascular

Up to 10% of combat wounds from current conflicts end in life threatening infection called sepsis

Mortality and cost of sepsis in 2009

	Warfighter	Civilian
Deaths	508	215,000
Cost	$40M	$16.7B

Preventable deaths from sepsis equal OIF/OEF casualties

Aim to reduce blood pathogen load by 90% in one day and reduce mortality by at least 20%

Save at least 43,000 US lives and $3.3B annually

Current limitations in treatment of sepsis

Poor Sensitivity

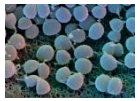
0.1% of blood volume cultured daily

Pathogen not determined in up to 50% of septic patients

Diagnose wound and blood infection by a gross change during an intermittent exam

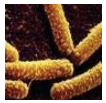
Unable to Scrub Pathogens

S. aureus



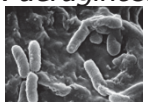
(0.6-0.7 µm)

K. pneumoniae



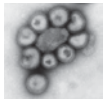
(0.3 x ~6 µm)

P. aeruginosa



(0.3 x ~6 µm)

Influenza



(80-120 nm)

Mortality increases with higher pathogen load

No effective method for removing pathogens and toxins from patient's blood

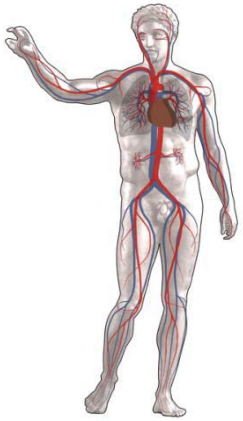
Ineffective Therapy

30% mortality with SOA treatment

Protocol-based antibiotic treatment continues as pathogen load and inflammation spiral out of control

New approaches in the treatment of sepsis

Sense Pathogens

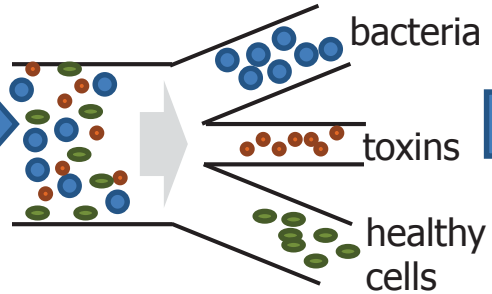


100% of blood volume interrogated continuously

Early identification of the presence of pathogen in wound and blood

Persistent interrogation of wound fluid and entire blood volume

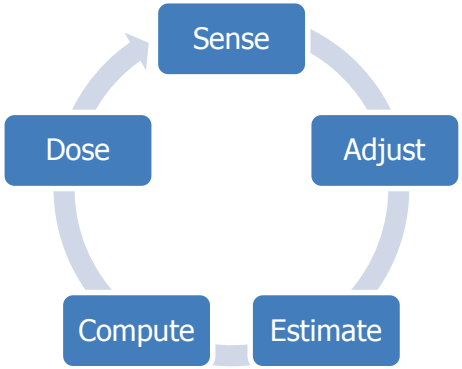
Scrub Pathogens and Toxins



Remove pathogen before sepsis develops

Continuous high volume removal of pathogens from blood without specialized binding chemistries or pathogen identification

Closed Loop Around Therapy



Monitor and maintain healthy state

Multivariable predictive control modulates key health parameters faster than underlying disease process

Technical challenges

Continuous sensing in biological fluids without biofouling

High flow microfluidic transport without activation or anticoagulant

High extraction efficiency from complex fluid across multiple size scales

Predictive models do not capture the complexity of biological systems

Technical challenges

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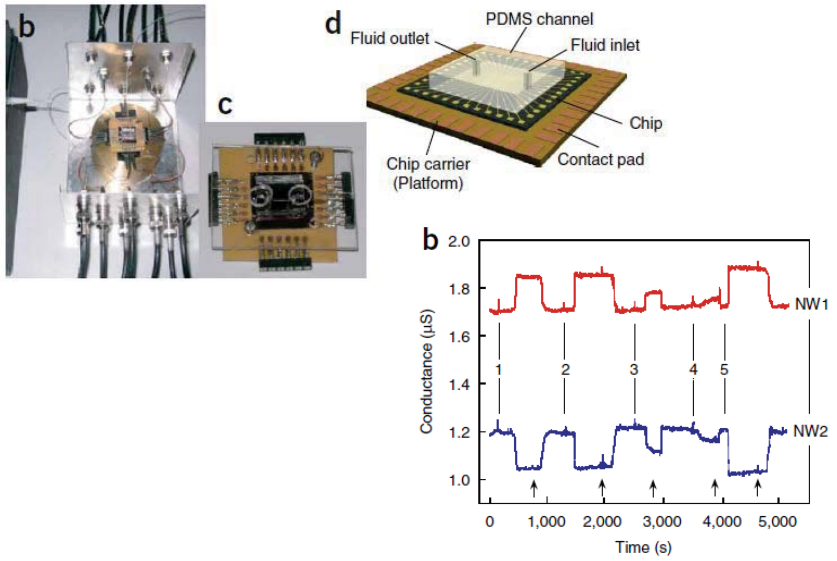
Predictive models do not capture the complexity of biological systems

Importance: Early detection of infection increases survival rate

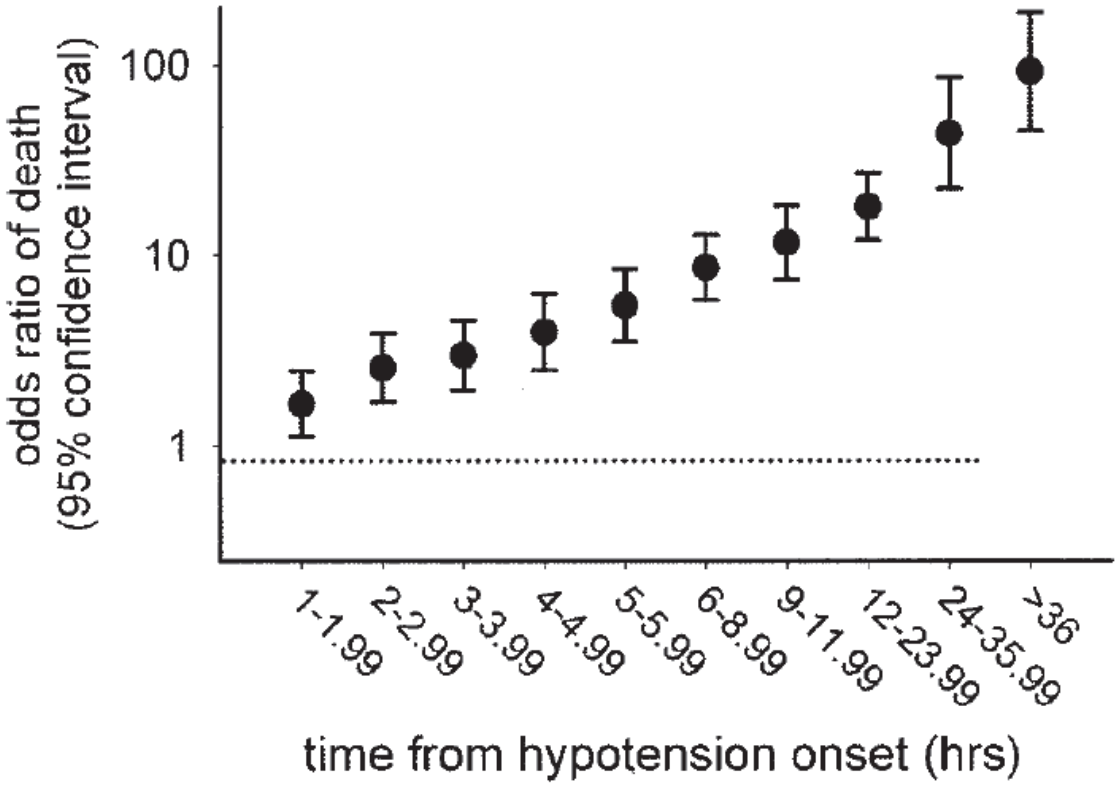
Current Limitation: Lack robustness to maintain performance over long periods of continuous use due to biofouling



Patolsky *et al.* Nat. Prot., 4 1711-1723, 2006

Delay in detection of sepsis increases mortality

Increased risk of mortality with delay in therapy



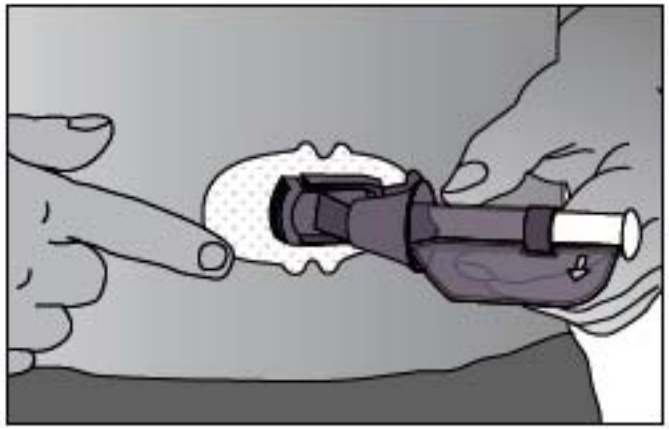
Kumar, Crit Care Med, 34 1589-1596, 2006

Continuous sensing in complex biologic fluids

- **Detect pathogens and/or biomolecules in blood**

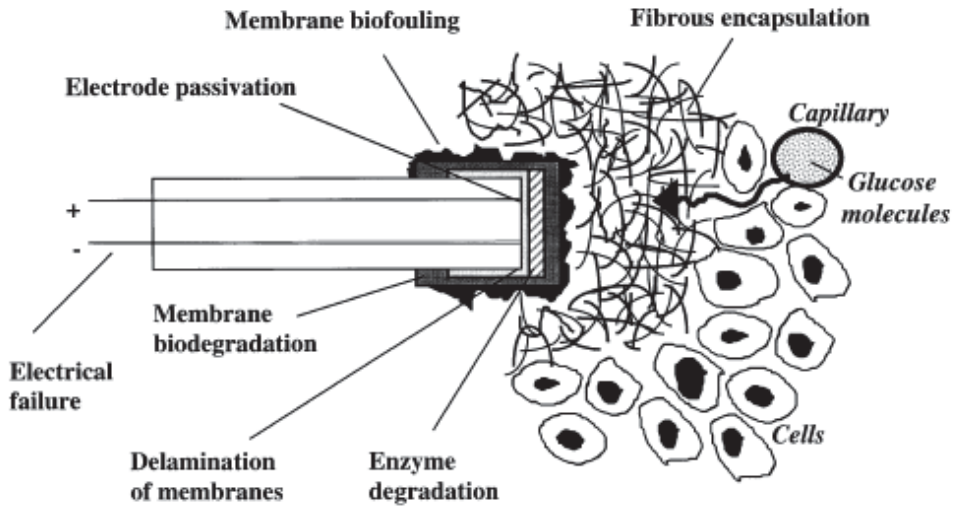
- **Identify and determine concentration (load) of pathogen, biomolecule, and/or activated cells in blood and wound fluid**

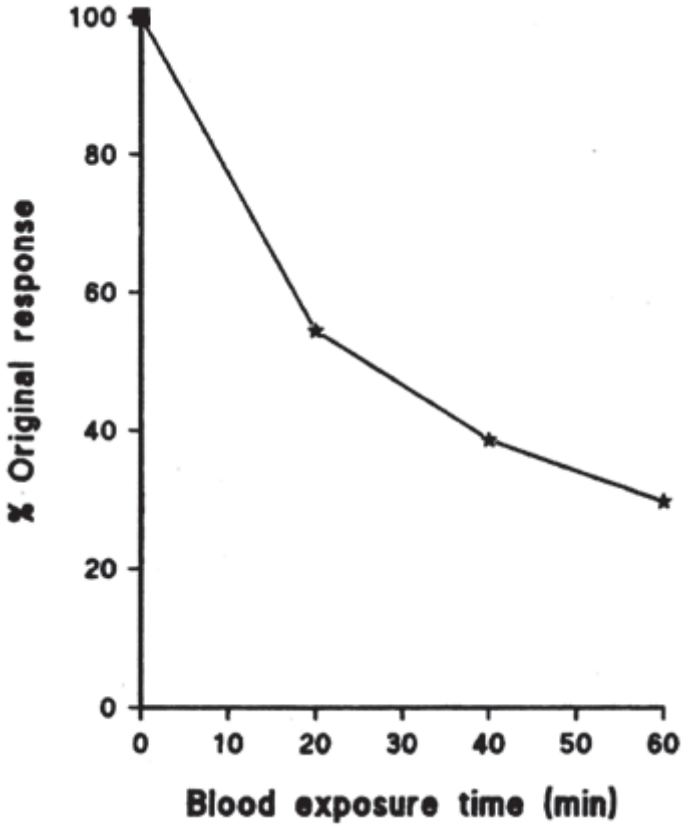
Indwelling glucose sensor FDA approved for 7 day use



Dexcom

Glucose sensor performance degradation over time

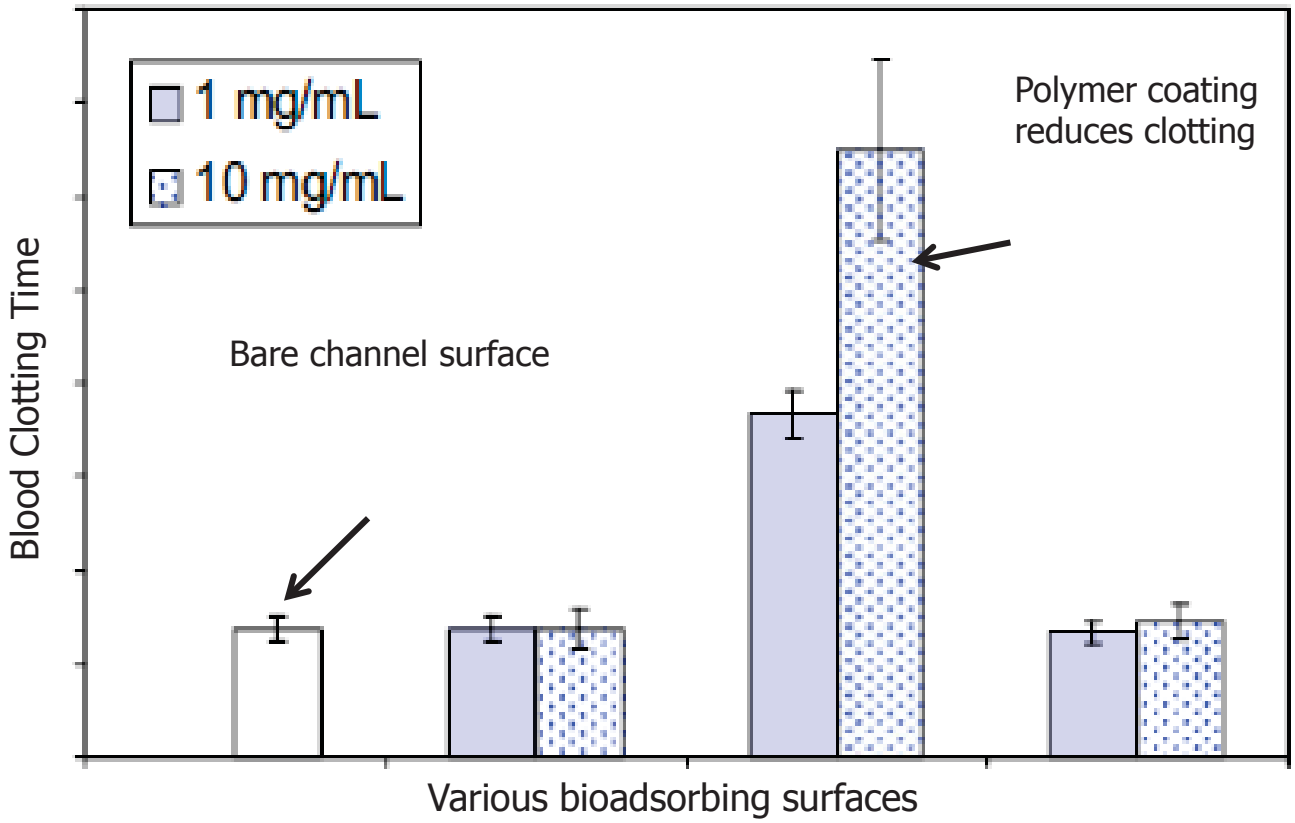




Wisniewski and Reichert, Colloids and Surfaces, 18 197-219, 2000
Reddy and Vagama, Sensor, 350 77-89, 1997

Coating to extend device lifetime > 24 hrs.

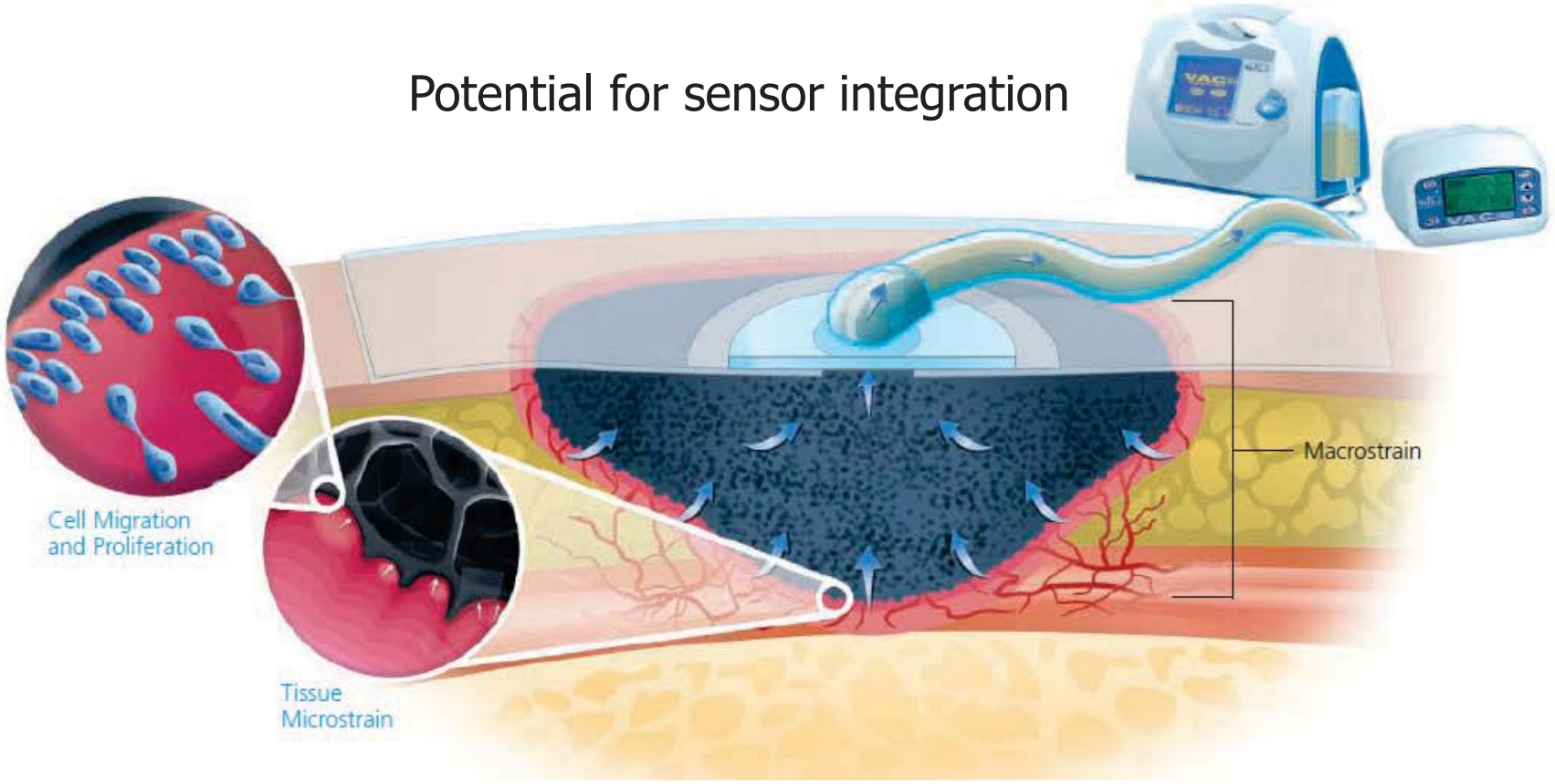
Surface modification is critical to extended use (> 24 hrs)



Zhang Z, Zhang M, Chen S, Horbett TA, Ratner BD, Jiang S. Biomaterials. 2008; 29(32):4285-4291

Vacuum assisted closure (VAC) device

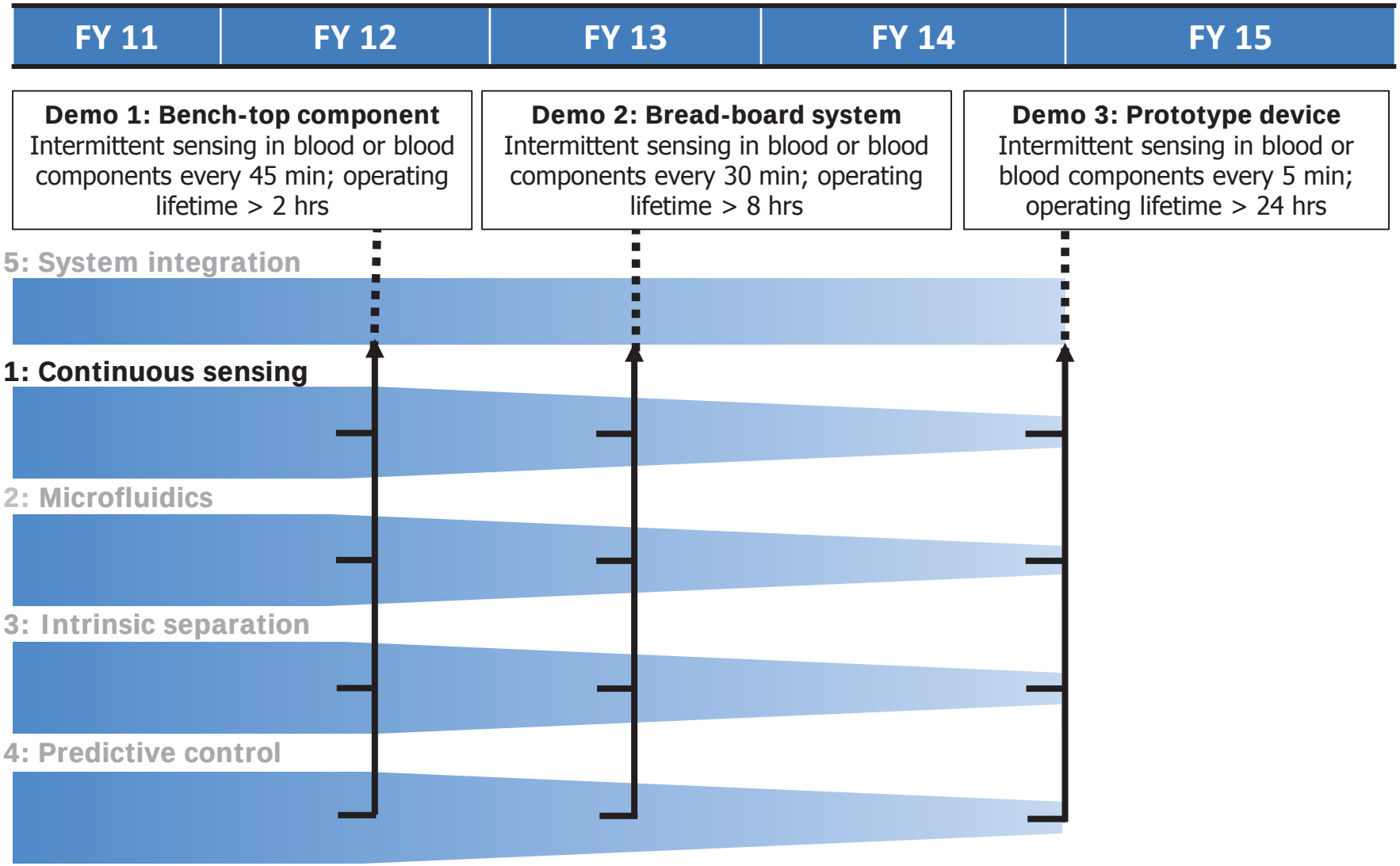
Potential for sensor integration



Microbiome and biomolecules –> colonization to wound infection to sepsis

KCI, Inc.

Sensing milestones



Technical challenges

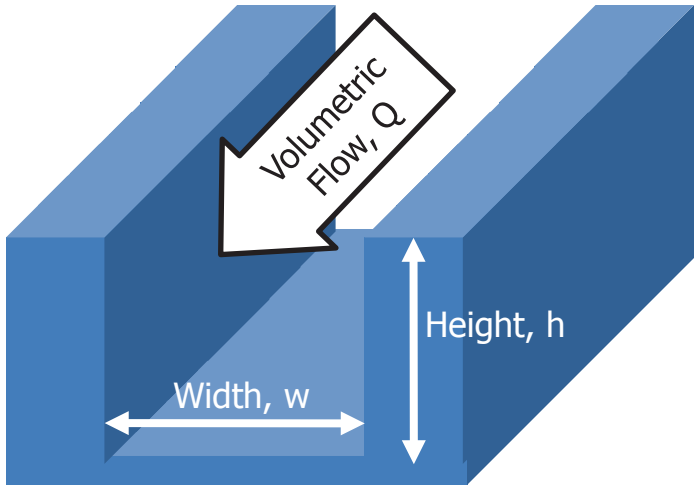
Continuous sensing in biological fluids without biofouling

High flow microfluidic transport without activation or anticoagulant

High extraction efficiency from complex fluid across multiple size scales

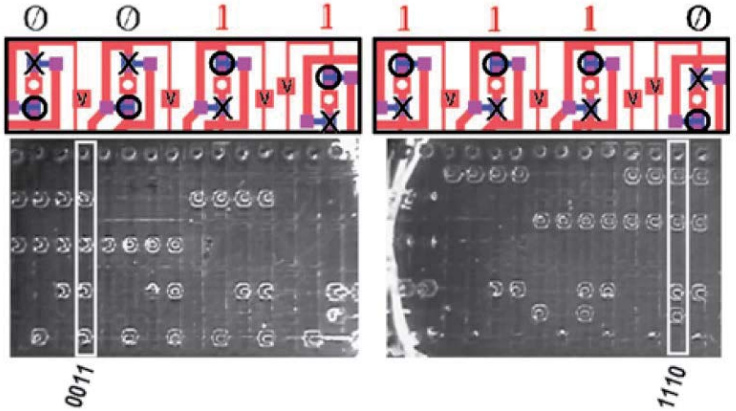
Predictive models do not capture the complexity of biological systems

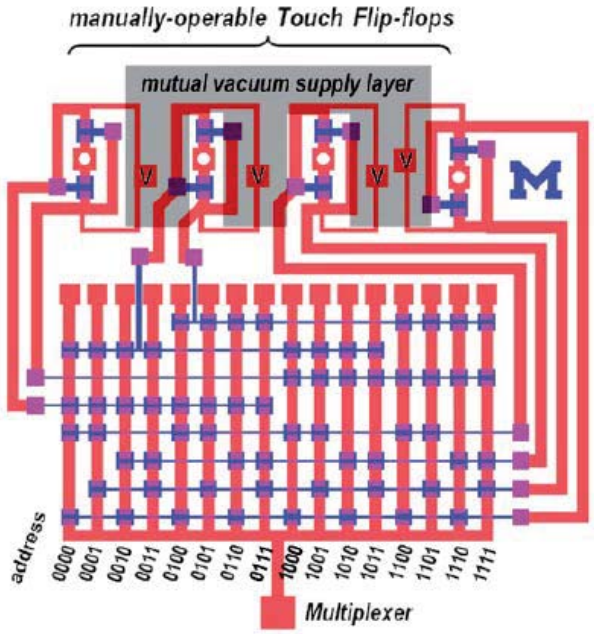
Importance:



Current Limitation: Blood clots within minutes without the addition of anticoagulants

Multiplexed microfluidic control

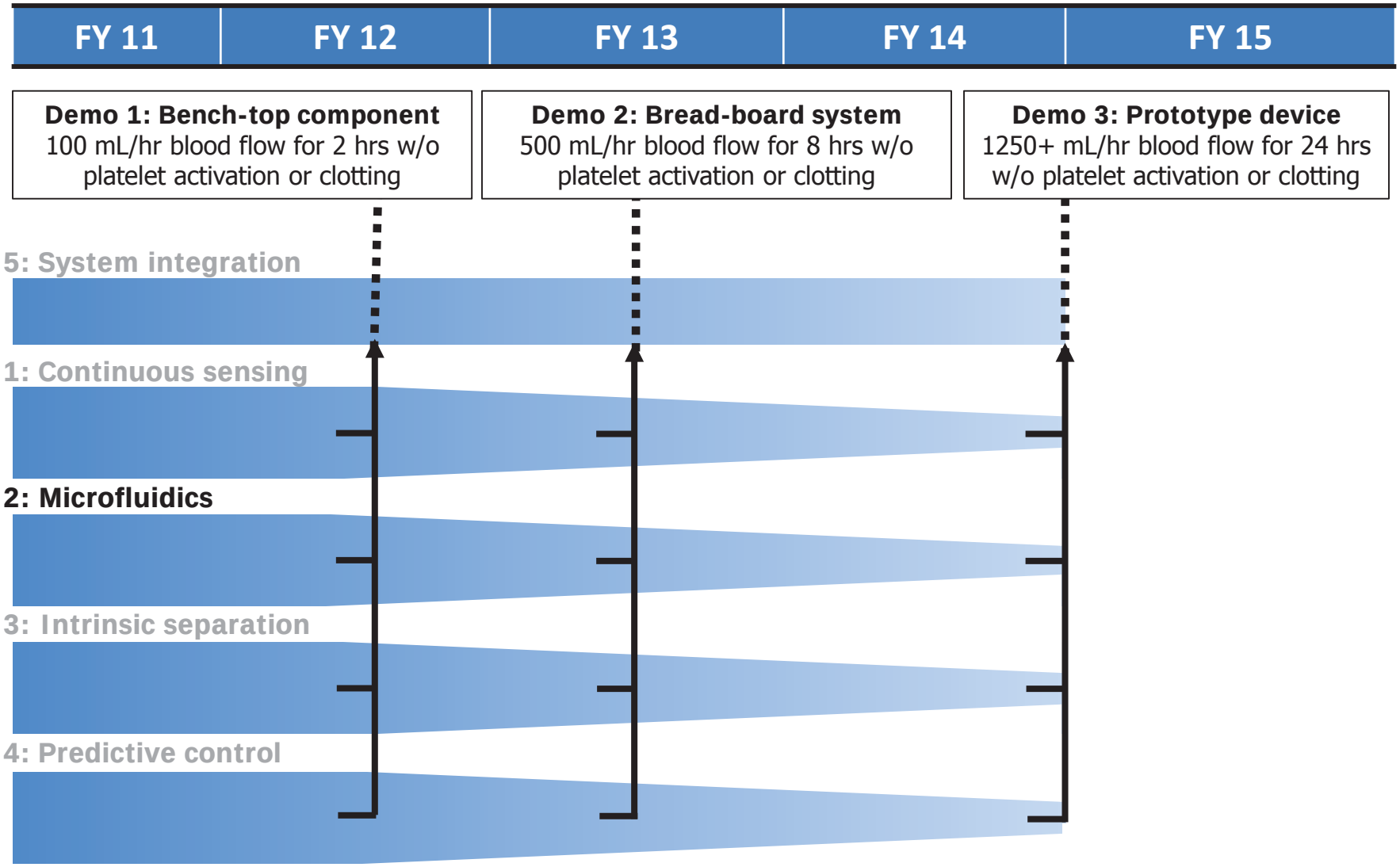




Multiplexed microfluidic device to generate sixteen independent outputs for multichannel manipulation and control

Rhee and Burns, Lab Chip, 9 3131-3143, 2009

Microfluidics milestones



Technical challenges

Continuous sensing in biological fluids without biofouling

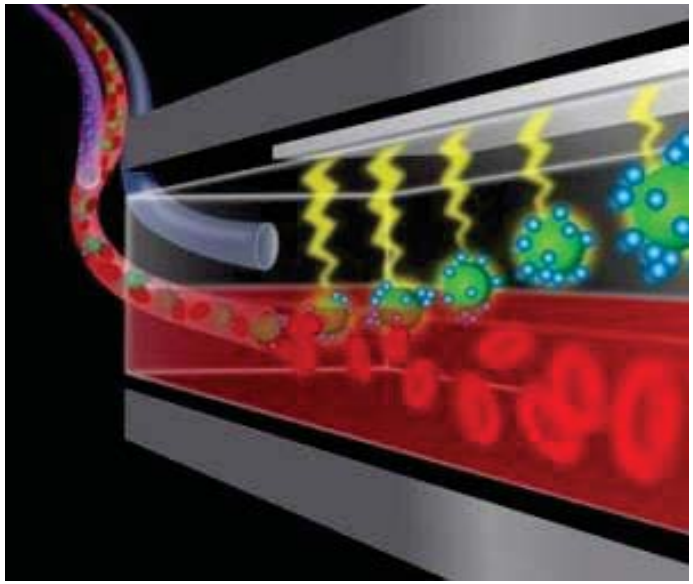
High flow microfluidic transport without activation or anticoagulant

High extraction efficiency from complex fluid across multiple size scales

Predictive models do not capture the complexity of biological systems

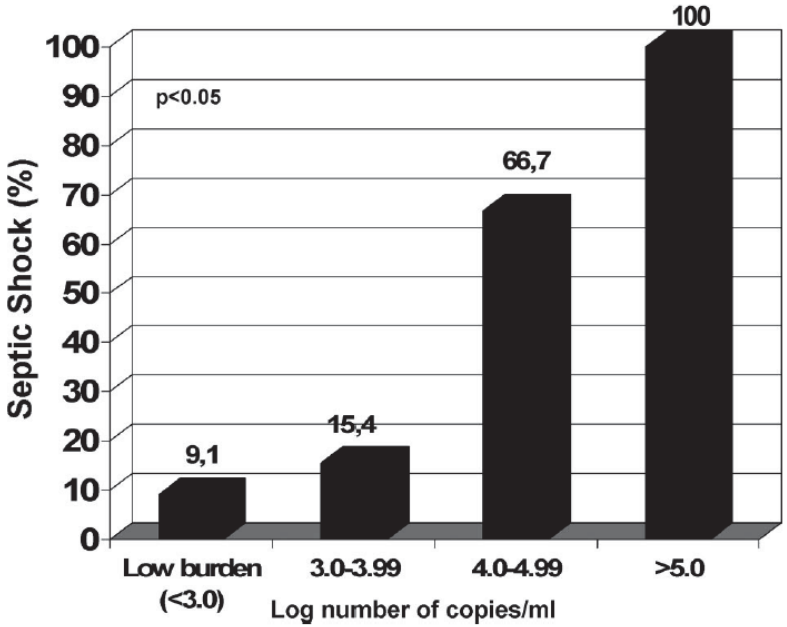
Importance: Need to separate objects that vary in size (10 nm – 10 μm) quickly (high flow rate) and effectively (high sieving coefficient)

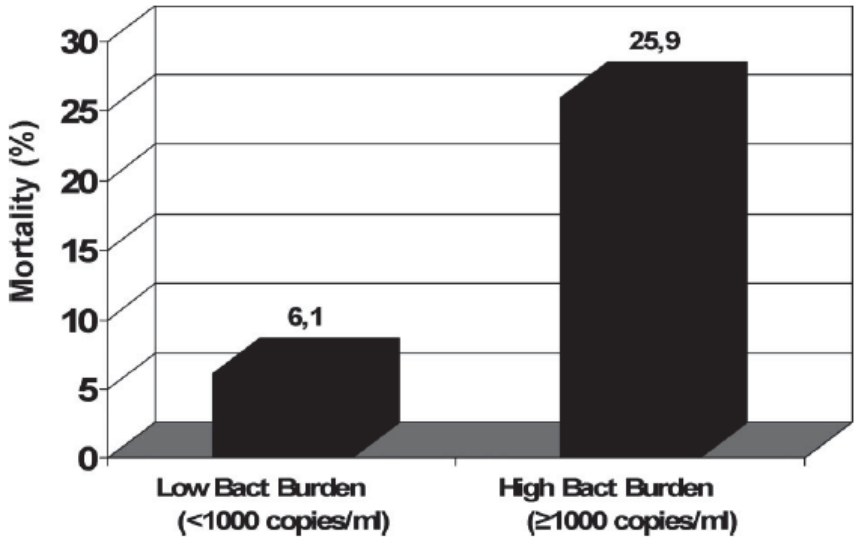
Current Limitation: Techniques are either selective or fast, but not *both*



Johnson and Yung, Children's Hospital Boston

Probability of sepsis increases with bacterial load

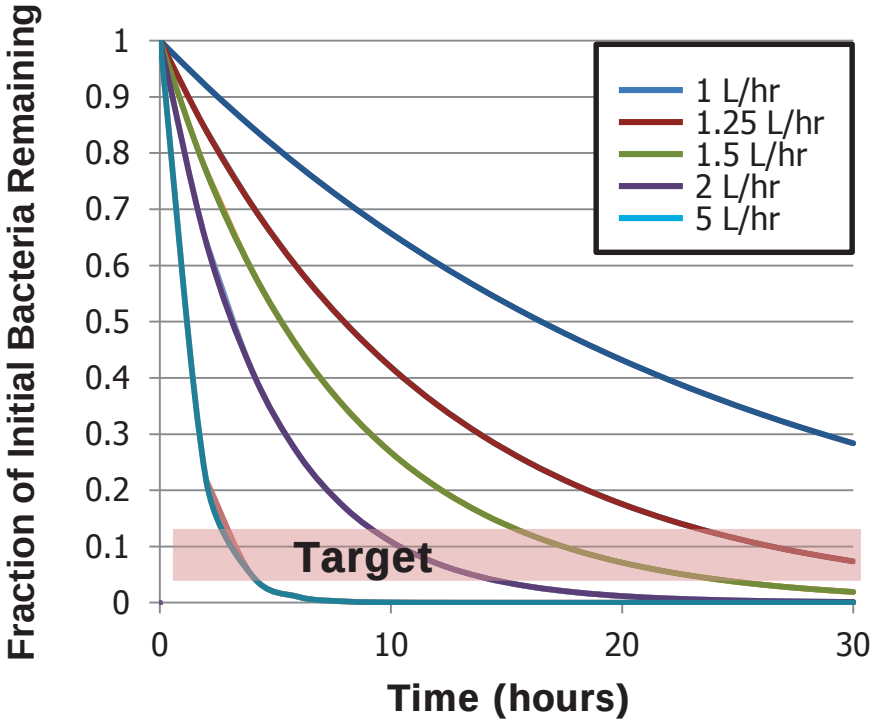




A 90% reduction in bacterial load leads to approximately a 20% decrease in the probability of septic shock across multiple log scales

Rello J et al. Chest 2009;136:832-840

Bacterial extraction needs to be effective and fast



Assumptions

- Extraction efficiency is > 90%.
- Must achieve a 90% clearance of starting material within one day.
- Bacteria doubling time is 300 minutes.

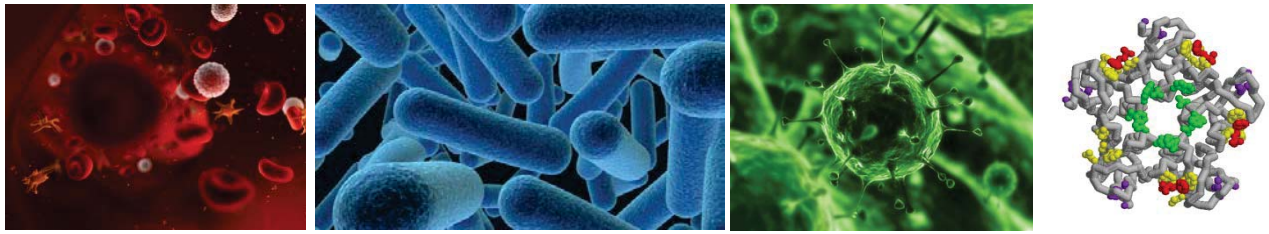
Success equates to a 20% decrease in sepsis and mortality

$$B_t = e^{(k - d - (f/v)*x) * t}$$

where B = fraction of initial bacteria; t = time (hr); k = growth rate (hr^{-1}); d = death rate (hr^{-1}); f = flow rate ($L*hr^{-1}$); v = total blood volume (L); x = extraction efficiency

Targets span orders of magnitude in size

Class	Size	Examples
Patient Cells	10 – 20 µm	Activated platelets, activated neutrophils, lymphocytes producing pro-inflammatory cytokines
Bacteria	1 – 5 µm	Clinical pathogens: *A. calcoaceticus-baumannii complex, K. pneumonia, P. aeruginosa, S. aureus;* Bio-agents: *B. anthracis, Y. pestis, F. tularensis*
Viruses	10 – 100 nm	Clinical pathogens: Hepatitis C, Human Immunodeficiency Virus, Influenza Bio-agents: Smallpox, viral hemorrhagic fevers
Toxins and biomolecules	10 – 100 nm	Aflatoxin, alpha toxin, amatoxin, botulinum toxin, endotoxin (e.g., lipopolysaccharide), ricin, Shiga toxin, tetanus toxin; cytokines (TNF-α, interleukins)



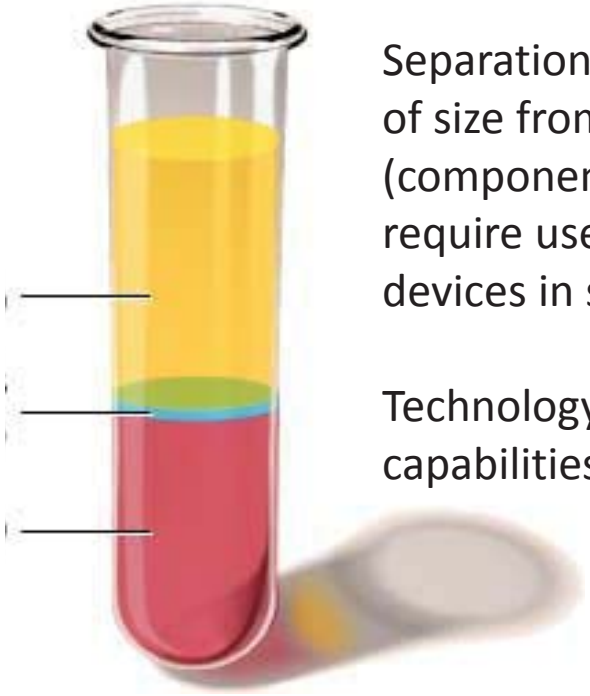
Must have label free techniques effective against a class of agents rather than a specific example

Blood components and targets

Plasma, virus , toxin (55%)

WBC, platelet, bacteria (<1%)

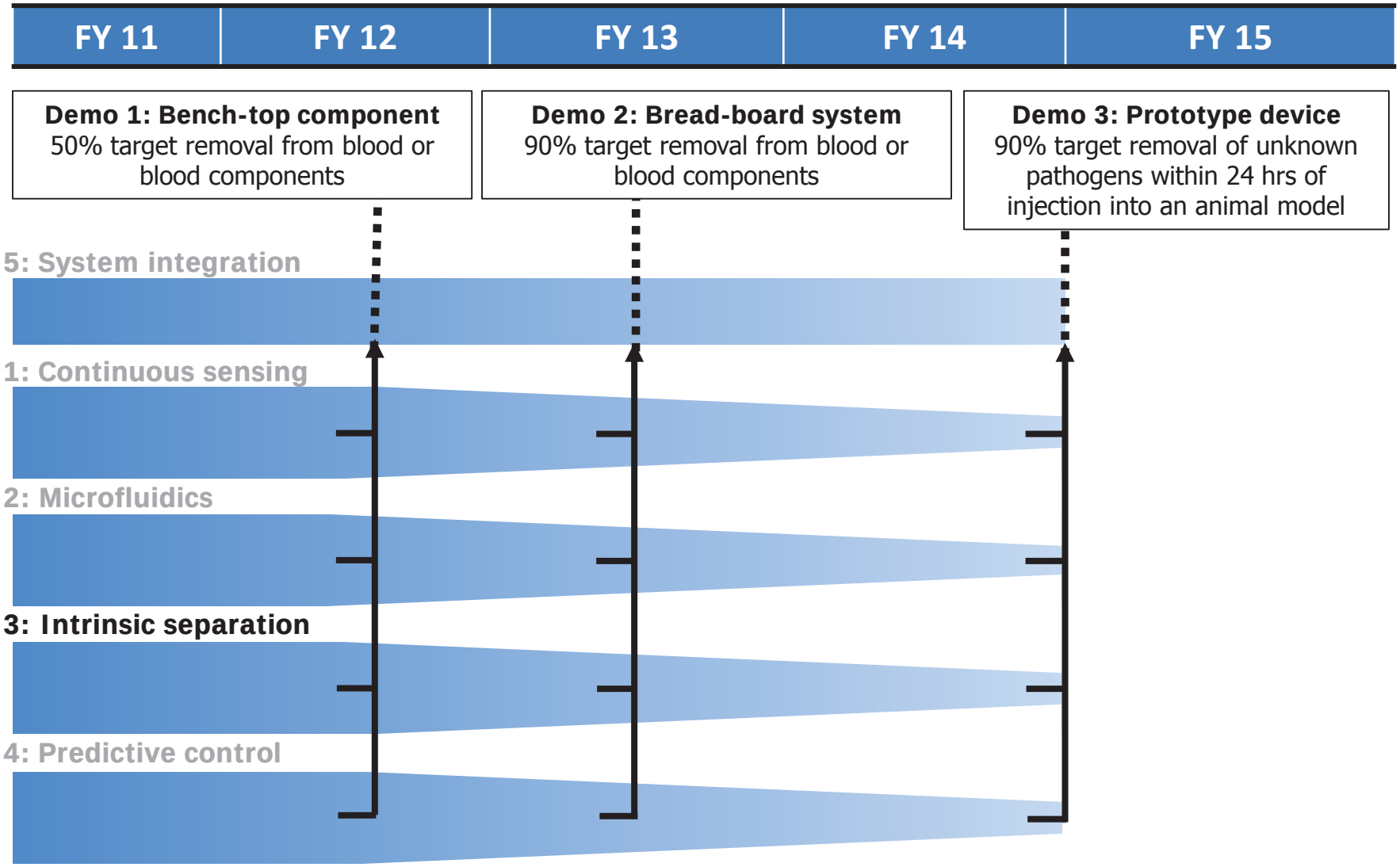
RBC (45%)



Separation of targets of a range of size from blood (components) is expected to require use multiple separation devices in series and parallel.

Technology agnostic and capabilities driven.

Separations milestones



Technical challenges

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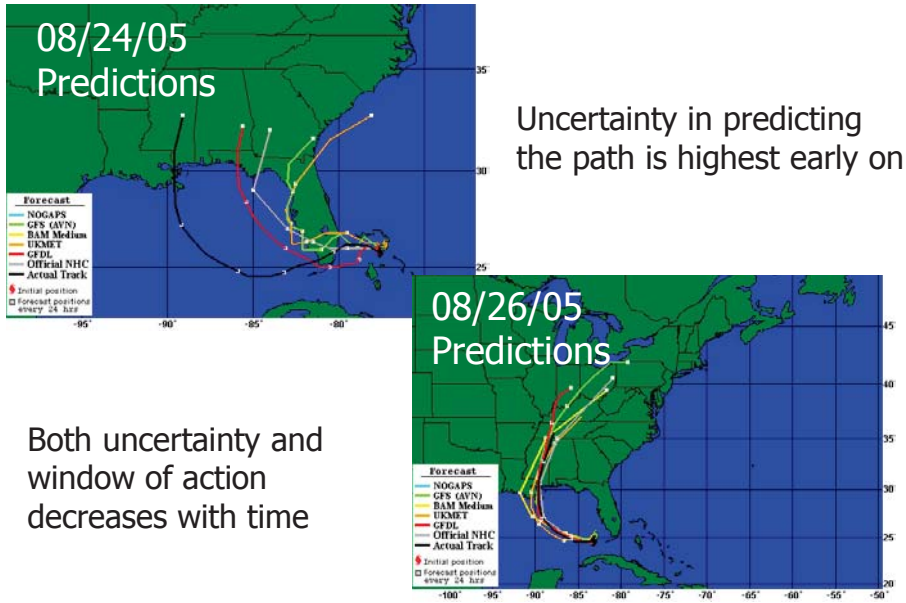
Predictive models do not capture the complexity of biological systems

Importance: Forecasting treatment is dependent on multiple patient specific variables

Current Limitation: High dimensionality complicates prediction in a dynamic environment with limited data

Weather forecasting analogy
actual data from Katrina



Uncertainty in predicting the path is highest early on

Both uncertainty and window of action decreases with time

Understanding sepsis through modeling and control

Many people have failed to accurately model sepsis –

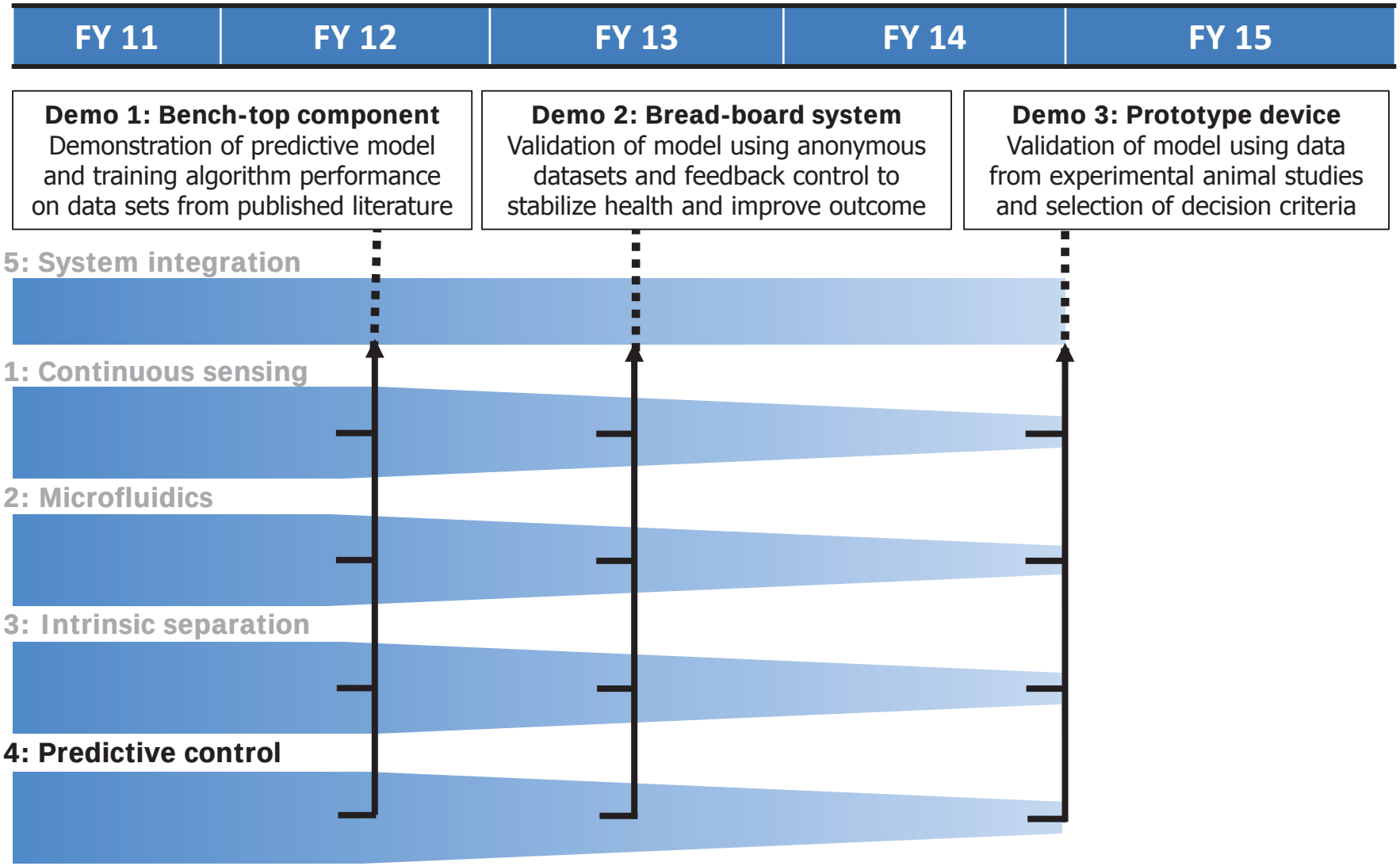
High dimensionality , dynamic environment , limited data

Although improved understanding of sepsis is beneficial, it is NOT the primary focus of this program.

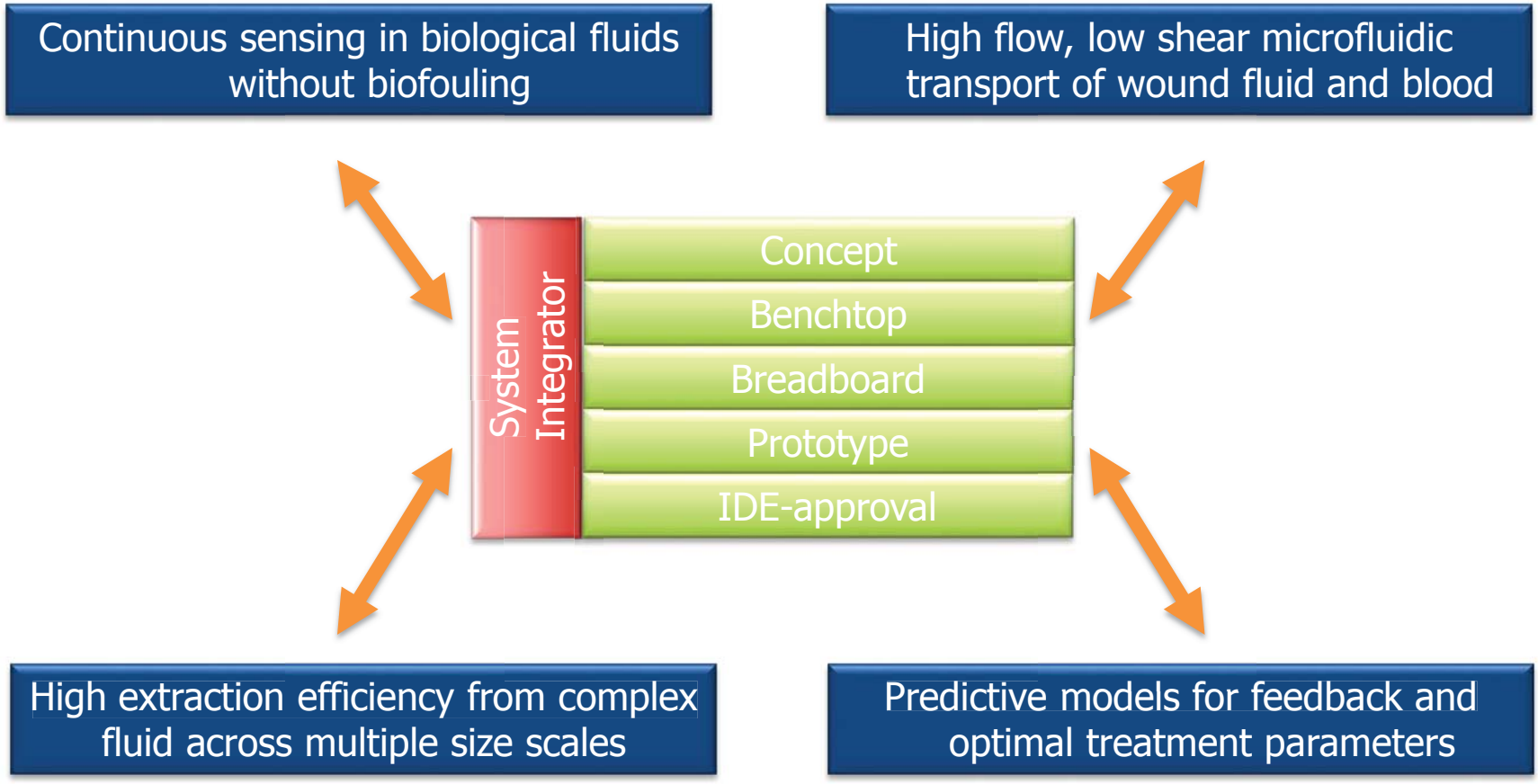
Dimensionality reduction and continuous model/control to

- **Determine target(s) of interest for sensing/separation (e.g. gene, biomolecule, cell)**
- **Close the loop on therapy (e.g. antibiotic therapy or activated cell removal)**
- **Refine model/control with continued use**

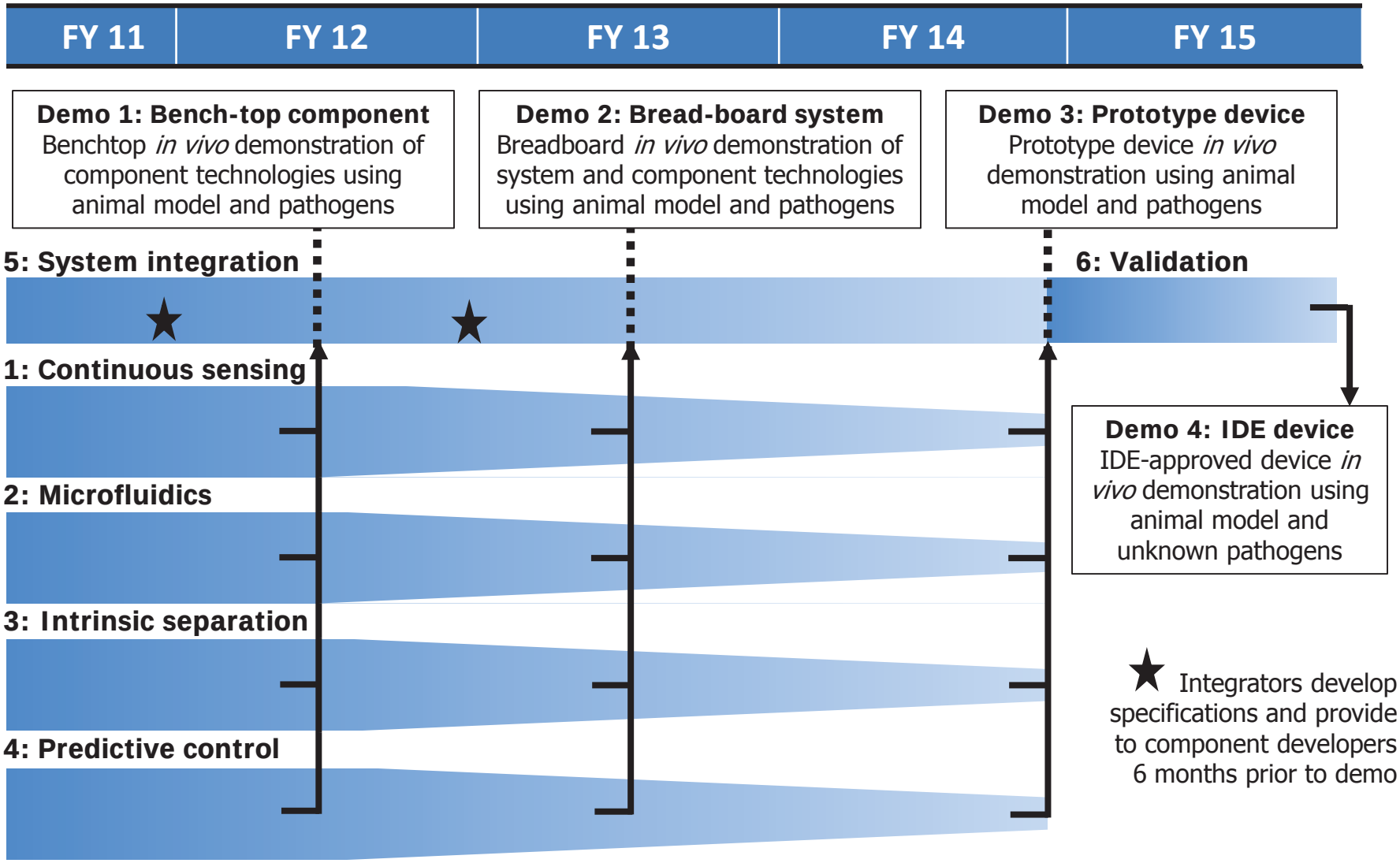
Modeling milestones



The role of a system integrator



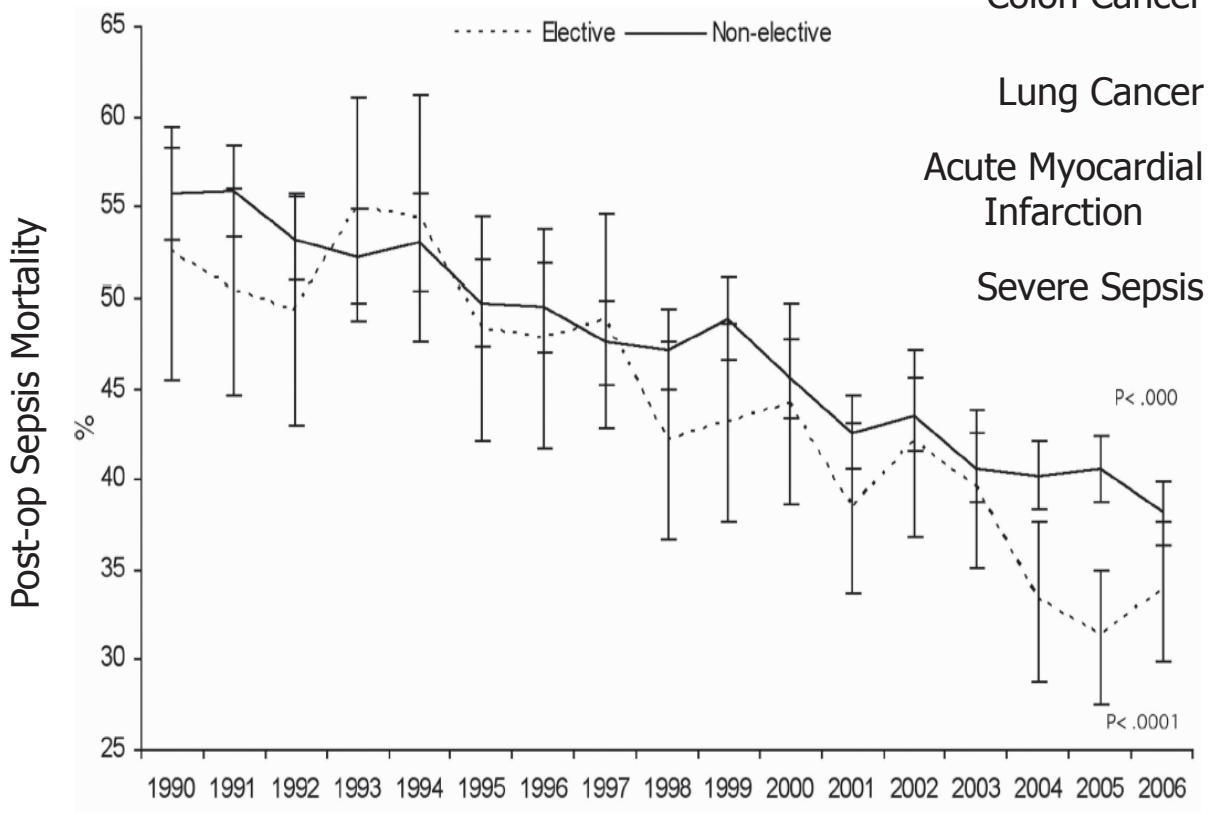
Integration milestones

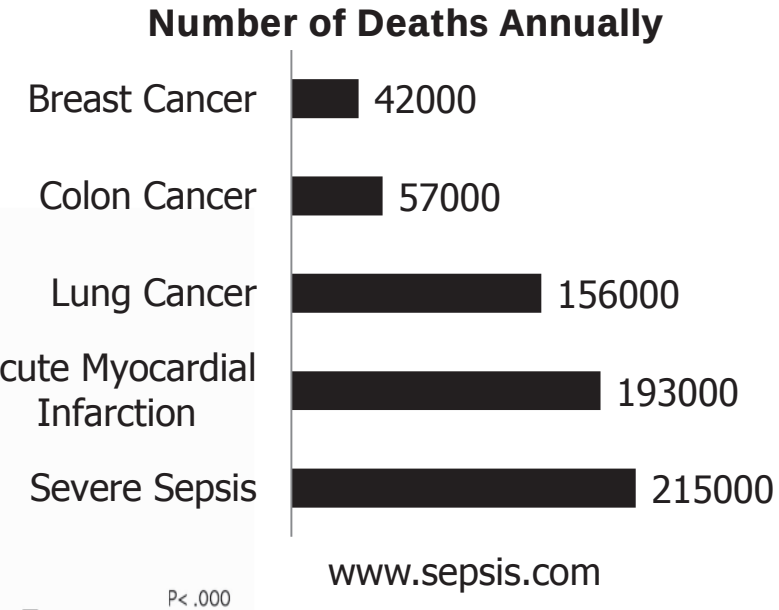


Backup Slides

www.darpa.mil

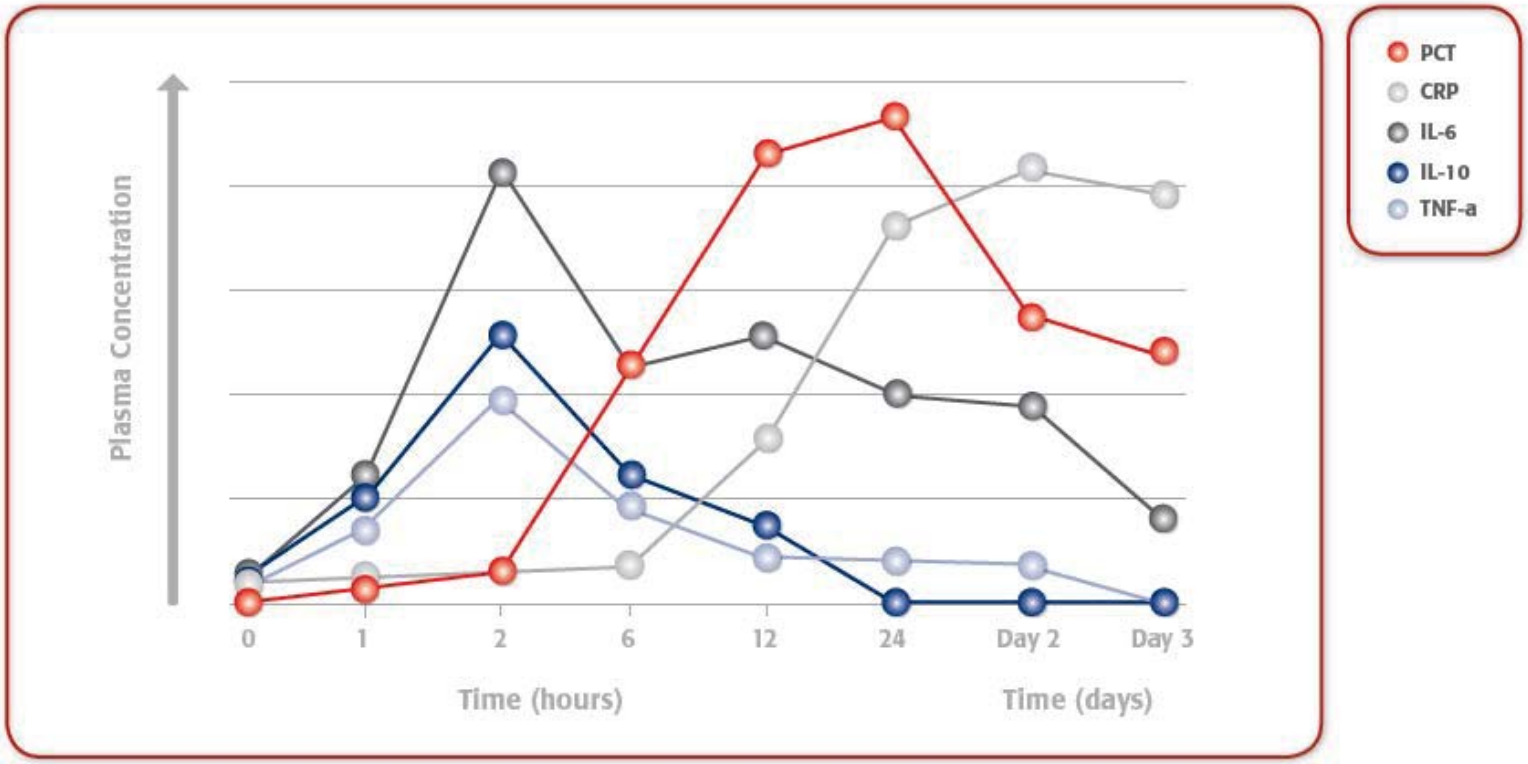
Need a revolutionary technology to combat sepsis





Sense and identify pathogens and biomolecules

Continuous sensing of multiple targets allow for accurate diagnosis



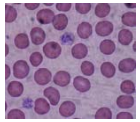
Biomerieux
Harbarth, Am J Respir Crit Care Med, 164 396-402, 2001

Species of interest

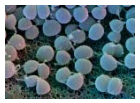
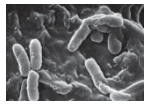
Name	MW
Tumor Necrosis Factor-α	17 kDa
Interleukins	40 kDa
Cytotoxins	50-70 kDa
Aflatoxin	300 Da
Hepatitis C	20 kDa

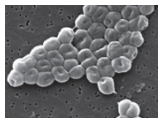
Blood targets

Activated host cells

Neutrophil	Basophil	Monocyte
		
(10-12 µm)	(12-15 µm)	(14-17 µm)
Eosinophil	**Lymphocyte**	**Platelet**
		
(10-12 µm)	(7-8 µm)	(2-3 µm)

Multidrug-resistant organisms

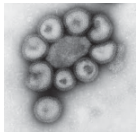
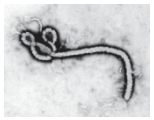
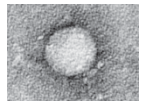
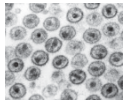
S. aureus	*K. pneumoniae*	*P. aeruginosa*
		
(0.6-0.7 µm)	(0.3 x ~6 µm)	(0.3 x ~6 µm)



Acinetobacter calcoaceticus-baumanii complex

(0.5-0.6 µm)

Viruses

Influenza	Ebola	Hepatitis C	HIV-1
			
(80-120 nm)	(80 x ~800 nm)	(55-65 nm)	(~120 nm)

Host inflammatory molecules

Eicosanoids

- leukotrienes
- prostaglandins
- prostacyclins
- thromboxanes

(< 1000 Da)

Cytokines

- IL-1
- IL-6
- TNF-α

(15 – 40 kDa)

Engineered pathogens

?

Toxins

Bacterial endotoxins

- lipopolysaccharide
- delta endotoxin

(10 kDa – 1 MDa)

Bacterial exotoxins

- tetanus
- alpha toxin
- Shiga toxin
- Cholera toxin

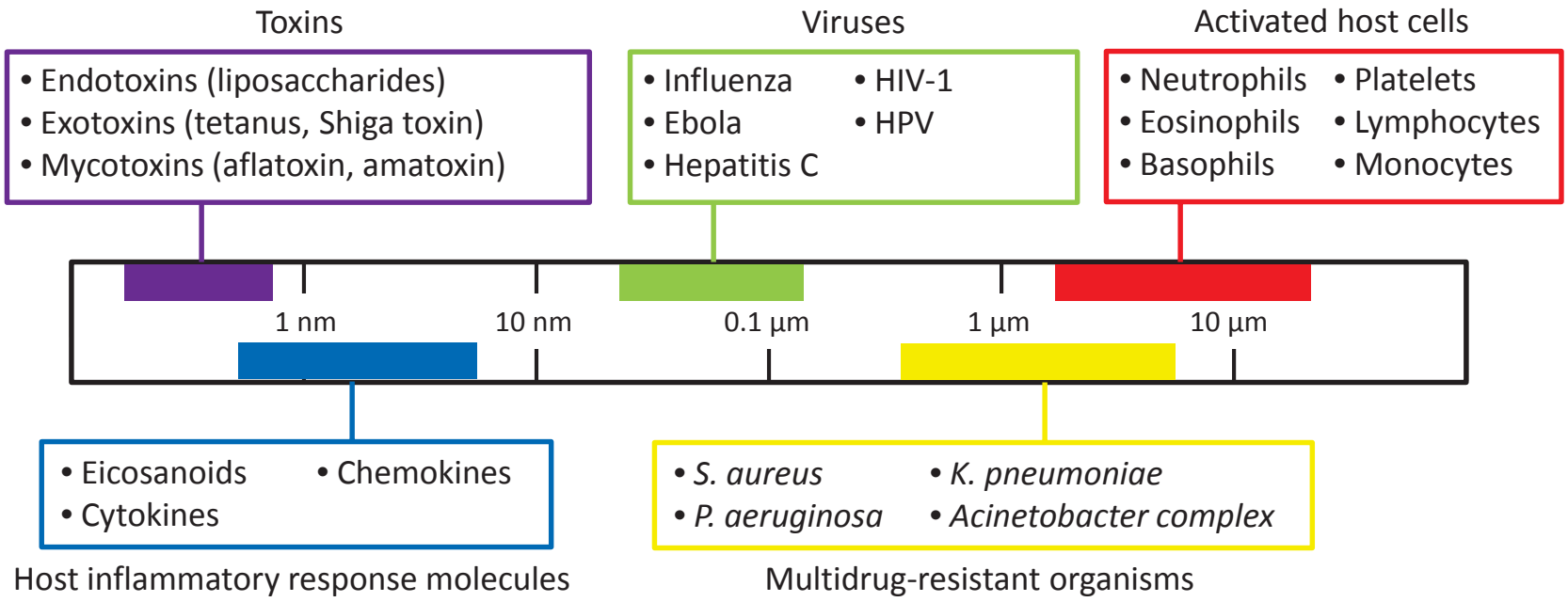
(< 200 kDa)

Fungal mycotoxins

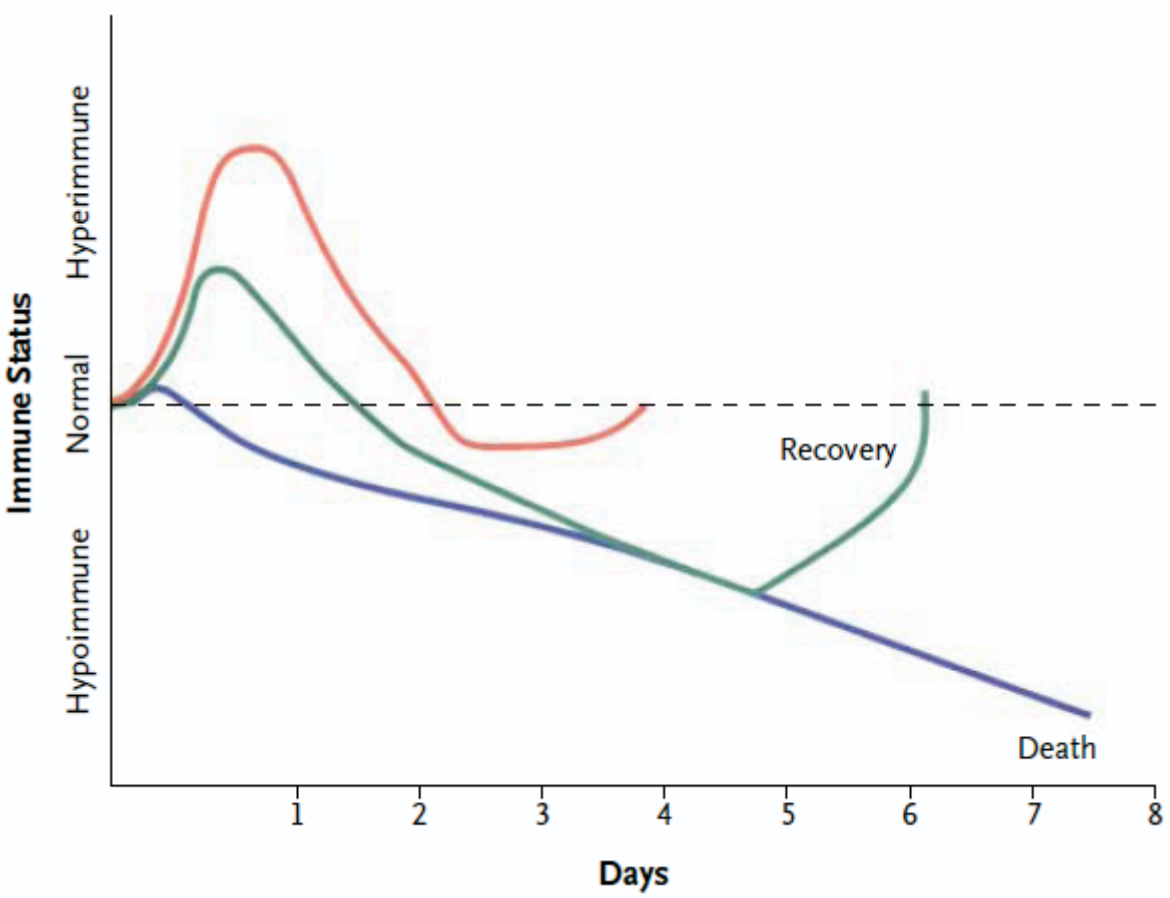
- aflatoxin
- amatoxin
- citrinin
- fumonisin

(< 1000 Da)

Blood targets



Body is hyperimmune during onset of infection



Hotchiss and Karl, NEJM, 348 138 – 150, 2003

Technical Task 1: Continuous sensing in complex fluid

This task will focus on the design and fabrication of pathogen and biomolecule sensors for continuous use in complex biologic fluids. Submissions should focus on the design of sensor materials, sensor coatings, and nonspecific surface detection. Resulting technologies should be able to sense pathogens and biomolecules in circulating blood, blood components and wound fluid at the limits of detection. Sensors addressing one or both of two related applications are encouraged. These applications are continuous intracorporeal sensing in circulating blood using indwelling vascular catheters and extracorporeal sensing in blood within the integrated device. Proposals to develop a sensor incorporated into a vascular catheter that detects the presence of a class of pathogen (bacteria or virus) or sepsis biomolecules in circulating blood or wound fluid are encouraged. The sensor must not foul during extended continuous use (i.e., days to weeks) and allow detection of target classes in circulating blood at very low concentrations. To ensure widespread use, a sensor applied as a coating on existing vascular catheters and enables non-invasive interrogation is desirable. Proposals to develop a sensor that resides within the integrated device to identify the class of pathogen (bacterial or viral), specific pathogens and biomolecules, and pathogen load are also encouraged. The primary performance objectives are resistance to biofouling and ability to detect pathogens and biomolecules at very low concentrations. Approaches that result in sensors with a limited life cycle (i.e. less than one day) are strongly discouraged. Approaches with small size, low weight, and efficient power consumption requirements are encouraged.

Technical Task 2: Biocompatible high-flow complex fluid manipulation

This task will focus on the design and fabrication of biocompatible high flow microfluidics to transport blood, blood components and other fluids within the integrated device. Channel and network designs that maximize flow rates while limiting shear are encouraged. Turbulent flow and stasis may be included in the microfluidic design for mixing or analysis provided there is no adverse effect on blood components. Use of non-fouling materials and coatings that do not result in contamination of the blood are strongly encouraged. Integrated digital control of the microfluidic network is strongly encouraged due to the multiple component technologies that will be used in series and parallel to optimize device performance. Since the final device blood flow rate target will be over a liter per hour, microfluidic design considerations allowing maximal use of interconnected networks and distribution of flow across many parallel channels is desired. The primary performance objectives are overall throughput, resistance to cellular activation/blood thrombosis, and resistance to adherence of pathogens/biofilm formation. Approaches that require anticoagulation of the blood are strongly discouraged.

Technical Task 3: Intrinsic separation from complex fluid

This task will focus on developing intrinsic separation technologies that remove harmful components of blood. The technologies will separate targets of interest from blood without the use of pathogen specific molecular labels or binding chemistries. Separation technologies effective against a target class (e.g. bacteria) as opposed to a specific target (e.g. *Staphylococcus aureus*) are encouraged. Targets for removal include pathogens (i.e., bacteria and viruses), relevant biomolecules (e.g., toxins and cytokines), and activated patient cells (e.g., neutrophils, lymphocytes, platelets). Table I.1. lists notable examples for each of these target classes. Although not required, proposers are encouraged to submit proposals that separate all classes of targets (i.e., pathogens, toxins and patient cells) from blood. It is expected that multiple separation technologies will be based in series and/or parallel to optimize extraction efficiency of target classes from various components of blood. Exceptional submissions to remove only a single class of targets from a component of blood (e.g., virus removal from plasma) will be considered.

The primary performance criteria for proposed separation technologies are extraction efficiency and flow rate. It is expected that system blood flow rates over one liter per hour will be required to clear at least 90% of pathogens from an adult human's blood volume within 24 hours. As continuous high flow rates are expected, separation technologies requiring administration of a single pass, non-recycled agent are discouraged. Approaches that introduce contaminants or activate blood components such that the blood cannot be returned to the patient are specifically excluded.

Inadvertent removal of essential blood components (e.g., red blood cells, leukocytes, proteins, fatty acids, cholesterol) should be minimized to prevent adverse clinical outcomes (e.g., symptomatic anemia, severe leukopenia). Proposers should list, quantify, and specify the clinical impact of loss of essential blood components expected with use of their proposed technology. Proposers are strongly encouraged to identify key risks in the proposed approach and include a detailed, well supported risk mitigation strategy that addresses the loss of essential blood components (e.g., red blood cell recovery from device effluent or leukocyte repletion by progenitor cells in bone marrow). Approaches that require anticoagulation of the blood are strongly discouraged, while those approaches that minimize size, weight, and power consumption are strongly encouraged.

Technical Task 4: Predictive modeling

This task will focus on establishing the mathematical foundations needed to manage sepsis on time scales relevant to resolving the underlying cause or causes. It is expected that realizing this goal will require appropriate combinations of predictive modeling and adaptive controllers. The developed formalism should account for measurements collected from different domains including hemodynamic, cellular, pathogenic, genomics/proteomics, and biochemical data. Furthermore, the formalism should be able to deal with the possibly high dimensional nature of some data sets (especially in the case of genomic/proteomic data), and should be able to capture temporal dependencies. Differences in disease progression due to pathogen macromolecular variability, host-pathogen interactions, inherent variability in patient pathophysiology, and impact of therapeutic interventions on the host should be incorporated. Proposed formalism should be aimed at identifying the measurements most informative for sepsis and patient state prediction, as well as the determining the time and type of intervention most likely to improve patient state. Similarly, the formalism should also aspire to describe the stage of infection and/or sepsis based on selected measurements and the optimal treatment protocol to improve patient health. Advances in a wide range of areas provide the expected basis for establishing the needed formalism. These include, but are not limited to, various statistical and machine learning techniques, such as parametric and nonparametric Bayesian approaches (*e.g.*, generalized Hidden Markov Models and latent factor analysis), multivariable model-based predictive controllers, granular dynamics, other adaptive feedback control techniques, biophysics, and open-far-from-equilibrium systems.

During Demo 1-3 the developed formalism should be compared to data from published literature. The formalism should be able to generalize or transfer learned knowledge to next phase involving anonymous patient data. These early studies should be followed by employment of anonymous patient data to demonstrate performance in the selection of decision criteria that may include choice of therapeutics, employment of DLT-based intervention for pathogen removal, or separation of hemodynamic, cellular or biochemical mediators linked to infection and sepsis progression. Proposers are required to have an existing data set that if awarded, will be shared with other performers to test newly developed models. Predictive power and robustness of the techniques developed during Demo 1-3 will be tested during the integration and validation phases (Technical Tasks 5-6), during which data will be collected from experimental animal studies. Strategies for validation through task 6 using data from experimental animal studies should determine which pathophysiologic measurements are relevant to assessing infection and sepsis, and intervention strategies examined during formalism development.

Technical Task 5: System integration

This task will focus on system design and integration of component technologies developed under Technical Tasks 1-4. Submitters should demonstrate past performance in leading a multi-team project of similar size, scope and complexity. Proposals should clearly define prior success and demonstrated understanding of the life-cycle approaches to systems integration.

The proposal should describe the design and development plan for the proposed device in detail. Submitters are encouraged to describe the proposed device throughout the development cycle (i.e., concept, bench-top component, bread-board system, prototype device, IDE-approved device, clinical product). Submitters are encouraged to design and fabricate the integrated device in-house as much as possible. Iterative end-user input throughout the development cycle is strongly encouraged (e.g., military medics, nurses, physicians, epidemiologists, etc).

Proposals should include a detailed plan for integration of component technologies that are developed within this program into the platform. The integration plan should clearly describe the demonstrations they will lead throughout the program. During these demonstrations, all component developers will demonstrate their technology using at least one platform provided by a system integrator. The integrator will develop and provide the required technical specifications to the component developers at least 6 months prior to the demonstration. Although not specifically encouraged, proposers to this task may also submit against other technical tasks and develop individual component technologies. Proposers must clearly breakout the program plan, budget, and technical approach for each task. Even if proposing against all tasks, integrators should define the process and cost of supporting additional component technology developed by separate performers under Tasks 1 – 4. The integrator will provide raw data and preliminary analysis to component developers within two weeks of each demonstration. The integrator will provide data, final analysis, and component developer comments to the program manager within four weeks of each demonstration. Proposals should provide rigorous protocols, justification, and approval for animal use.

Submitters should provide a detailed plan to obtain military and Food and Drug Administration (FDA) approval for use of the proposed medical device and a strategy for implementation throughout the military health system. Proposer will establish design and quality control plans specific to this program for submission to relevant regulatory agencies. As the lead systems integrator, it is expected that this task requires active engagement with the FDA Center for Device and Radiologic Health (CDRH) program officer serving as an advisor to this program. It is expected that the integrator will cooperate with members of Federally Funded Research and Development Centers for independent assessment and aid in the development of technology as indicated. Proposals should include a detailed commercialization plan including transition of the IDE-approved device to clinical trials within military medical commands and civilian sector after the completion of this program. Proposers should demonstrate prior success in facilitating agreements and protecting intellectual property developed within collaborative research and development efforts.

If the proposer is not currently approved for animal use, the submission should detail the plan for obtaining appropriate testing protocols and approval within 12 months of award.

Technical Task 6: System validation

This task will be a follow on to Technical Task 5 in which proposers will evaluate the device safety and establish efficacy using a suitable experimental animal exposed to common clinical pathogens and bio-agents. Proposals should provide rigorous protocols, justification for the use of proposed experimental animal system, and documentation of approval for animal use. The proposal should outline the plan for military and FDA regulatory approval including IDE-approval at the completion of this task. The proposal should define plans for transition of the device to military medical commands and civilian sectors for clinical trials after completion of this task and program. As in Technical Task 5, all proposers are required to have in place or detail the plan for obtaining the appropriate animal testing protocols and approval as required under DoD Regulations (DoD Instruction 3216.01).

Program objectives, milestones, and metrics

Task	Objective	Milestone	Metric
1: Continuous sensing in complex fluid	Continuously sense the presence of pathogens and biomolecules in flowing blood, blood components and wound fluid at state of the art limit of detection	Demo 1	Intermittent target sensing in blood or blood components measured at least every 45 minutes; single sensor operating time of 2 hours
		Demo 2	Intermittent target sensing in blood or blood components measured at least every 30 minutes; single sensor operating time of 8 hours
		Demo 3	Intermittent target sensing in blood or blood components measured at least every 5 minutes; single sensor operating time of 24 hours
2: Biocompatible high-flow complex fluid manipulation	Flow blood at greater than 1250 mL/hr with no cellular activation or thrombosis	Demo 1	100 mL/hr microfluidic system blood flow for at least 2 hours without platelet activation or clotting
		Demo 2	500 mL/hr microfluidic system blood flow for at least 8 hours without platelet activation or clotting
		Demo 3	1250+ mL/hr system blood flow for at least 24 hours without platelet activation or clotting
3: Intrinsic separation from complex fluid	Separate unknown pathogens and bio-agents from blood with greater than 90% extraction efficiency during the first 24 hours of purification	Demo 1	50% target separation from blood or blood components
		Demo 2	90% target separation from blood or blood components
		Demo 3	Removal of 90% of unknown pathogens within 24 hours of injection in an animal model
4: Predictive modeling and control	Model patient health with sufficient forecasting to predict changes in stability and optimal therapy for improving patient state	Demo 1	Demonstration of a clinically relevant sepsis predictive model and training algorithm performance on data sets from published literature
		Demo 2	Validation of the sepsis predictive model using larger anonymous clinical datasets and demonstrated performance in selection of decision criteria and feedback control to stabilize health and improve outcomes
		Demo 3	Validation of the sepsis predictive model using data derived from experimental animal studies and demonstrated performance in the selection of decision criteria related to sepsis assessment and interventions
5: System integration	Integrate multiple component technologies into a functioning blood purification system with initial tests in a large animal system	Demo 1	Bench-top *in vivo* demonstration of component technologies (with respective stated metrics) using a relevant small animal model and pathogens selected from Table I.1
		Demo 2	Bread-board *in vivo* demonstration of system and component technologies (with respective stated metrics) using a relevant small animal model and pathogens selected from Table I.1
		Demo 3	Prototype device (with component capabilities dictated by their respective stated metrics) *in vivo* demonstration using a relevant large animal model and pathogens selected from Table I.1
6: System validation	Validate the integrated system with a series of animal tests that lead to IDE-approval by FDA	Demo 4	IDE-approved device (with component capabilities dictated by their respective stated metrics) *in vivo* demonstration using a relevant large animal model and unknown pathogens / bio-agents provided by the government