Disruptive Innovation for Next Generation Biomanufacturing

Govind Rao
Workshop on Innovations in Biomanufacturing
Feb 27, 2020

CoI: IP

CAST
Center for Advanced Sensor Technology

UMBC

Disruptive Innovations not there in 2000

- iPhone
- iPad
- Facebook
- 4G
- Uber
- Airbnb
- Android
- Film>>Digital
- Tesla
- Netflix (streaming)
- Instagram
- Snapchat
- WhatsApp
- AWS
- Google, Cloud meant something else!

WHERE IS UMBC?

Low-Cost Microbioreactor for High-Throughput Bioprocessing

Yordan Kostov, Peter Harms, Lisa Randers-Eichhorn, Govind Rao

Department of Chemical and Biochemical Engineering, University of Maryland Baltimore County, Baltimore, Maryland 21250; telephone: (410) 455-3415; fax: (410) 455-1049; E-mail: grao@umbc.edu and Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, Maryland 21201-1503

Received 1 July 2000; accepted 17 October 2000

Abstract: The design of a microbioreactor is described. An optical sensing system was used for continuous measurements of pH, dissolved oxygen, and optical density in a 2 mL working volume. The K_La of the microbioreactor was evaluated under different conditions. An *Escherichia coli* fermentation in both the microbioreactor and a standard 1 L bioreactor showed similar pH, dissolved oxygen, and optical density profiles.

The low cost of the microbioreactor, detection system, and the small volume of the fermentation broth provide a basis for development of a multiple-bioreactor system for high-throughput bioprocess optimization. *Biotechnol Bioeng* **72:** 346–352, 2001.
Keywords: microbioreactor; optical sensing; fermentation

of operational/nutritional parameters on cell growth and product formation in a systematic and statistically significant manner. Currently available instrumented bioreactors are expensive and bulky, thus making bioprocess development inefficient as large numbers of simultaneous experiments simply cannot be conducted. Recent advances in optical sensor technology provide a possible solution.

Traditionally, bioprocess technology has been critical to the development and availability of new drugs and vaccines. Additionally, bioprocesses are important in a wide variety of industries besides pharmaceuticals—food industry, ecology, water treatment, etc. (Arroyo et al., 2000; Bakoyianis

CAST Core Competence: Innovative Sensor Technologies

What? Disruptive Innovation leading to Paradigm Shifting Practices

Why? Reduce Healthcare Costs

How? Low-cost Integrated Opto-electro-bio-mechanical Devices

MLK: Life's most persistent and urgent question is, 'What are you doing for others?'

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AN HONORS UNIVERSITY IN MARYLAND

Norm Augustine, Author of Augustine's laws

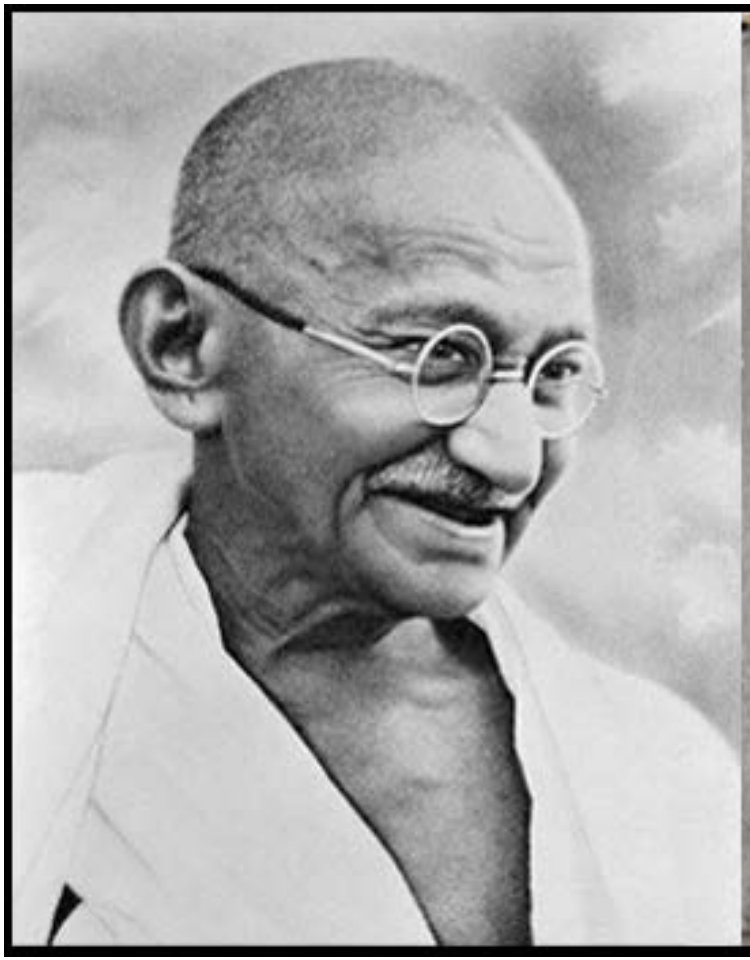
Per capita health spending is now >$12,000, or $3.85 trillion in 2018, almost certain to cross $4 trillion for 2019.

- Of the **$3.3 trillion** spent on healthcare in 2016, **$450 billion** was spent on pharmaceuticals, including rebates*
- Biologics: **2%** of all US prescriptions but **37%** of net drug spending and over **90%** of net growth in drug spending*
- 1 in 4 patients report difficulty in affording their medicine**
- Patients can pay thousands of dollars out of pocket for specialty tier drugs, even with insurance coverage

*2017, IQVIA
**2019 KFF Health Tracking Poll

Augustine Equation: Cost, as a fraction of GDP (in percent) = 0.25 Y—487, where Y is the calendar year of interest. In 2018, 18.5% of GDP was spent on healthcare

WE CAN AND MUST FIX THIS HERE AND NOW!



GANDHIJI'S TALISMAN

"I will give you a talisman. Whenever you are in doubt or when the self becomes too much with you, apply the following test :

Recall the face of the poorest and the weakest man whom you may have seen and ask yourself if the step you contemplate is going to be of any use to him. Will he gain anything by it ? Will it restore him to a control over his own life and destiny ? In other words, will it lead to Swaraj for the hungry and spiritually starving millions ?

Then you will find your doubts and your self melting away."

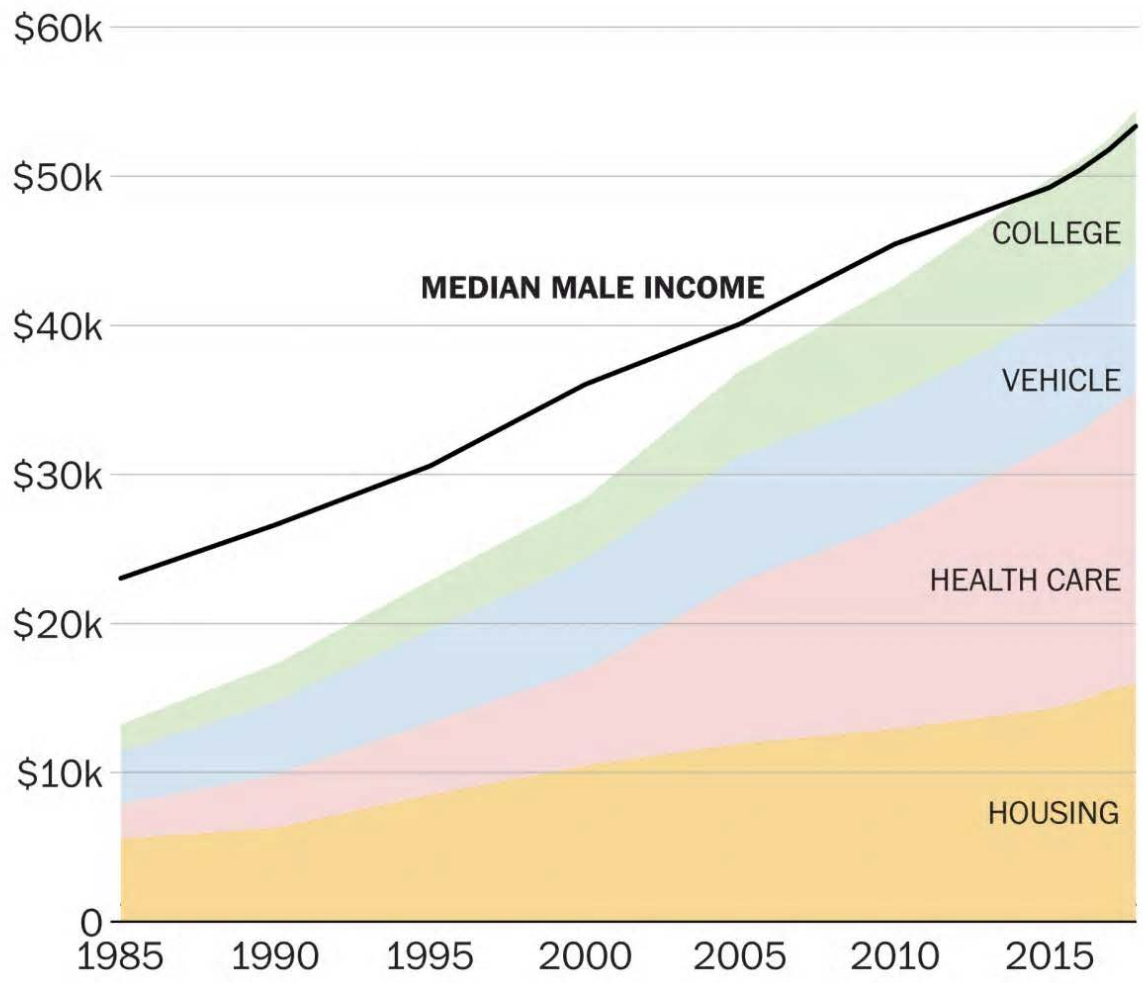
M K Gandhi

A year of wages no longer covers a year of family expenses

US healthcare

Major annual household expenditures for a family of four vs. median male income, 1985–2018

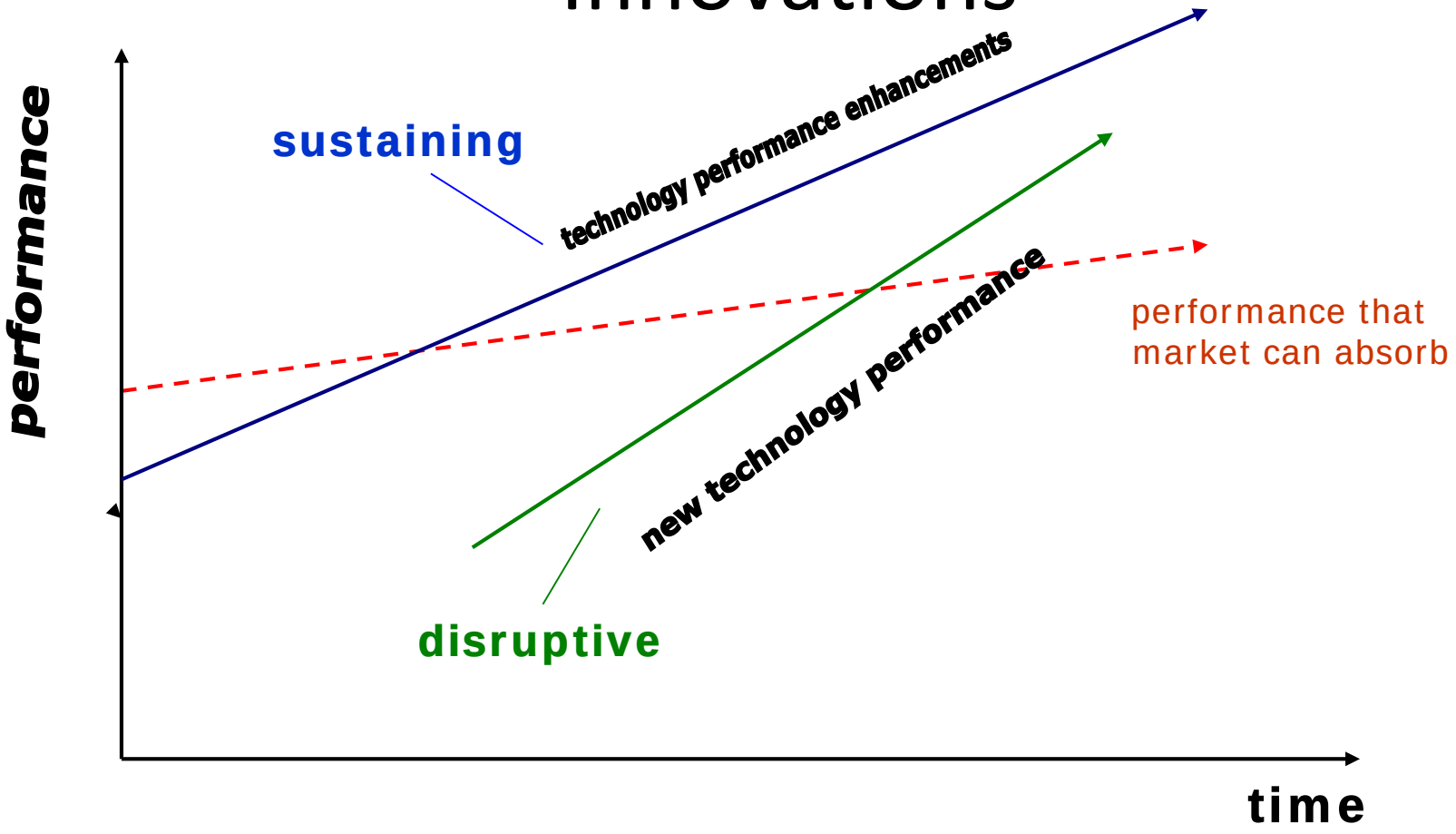
Michael Sainato
Thu 14 Nov 2019 02.00 EST



Source: The Cost-of-Thriving Index

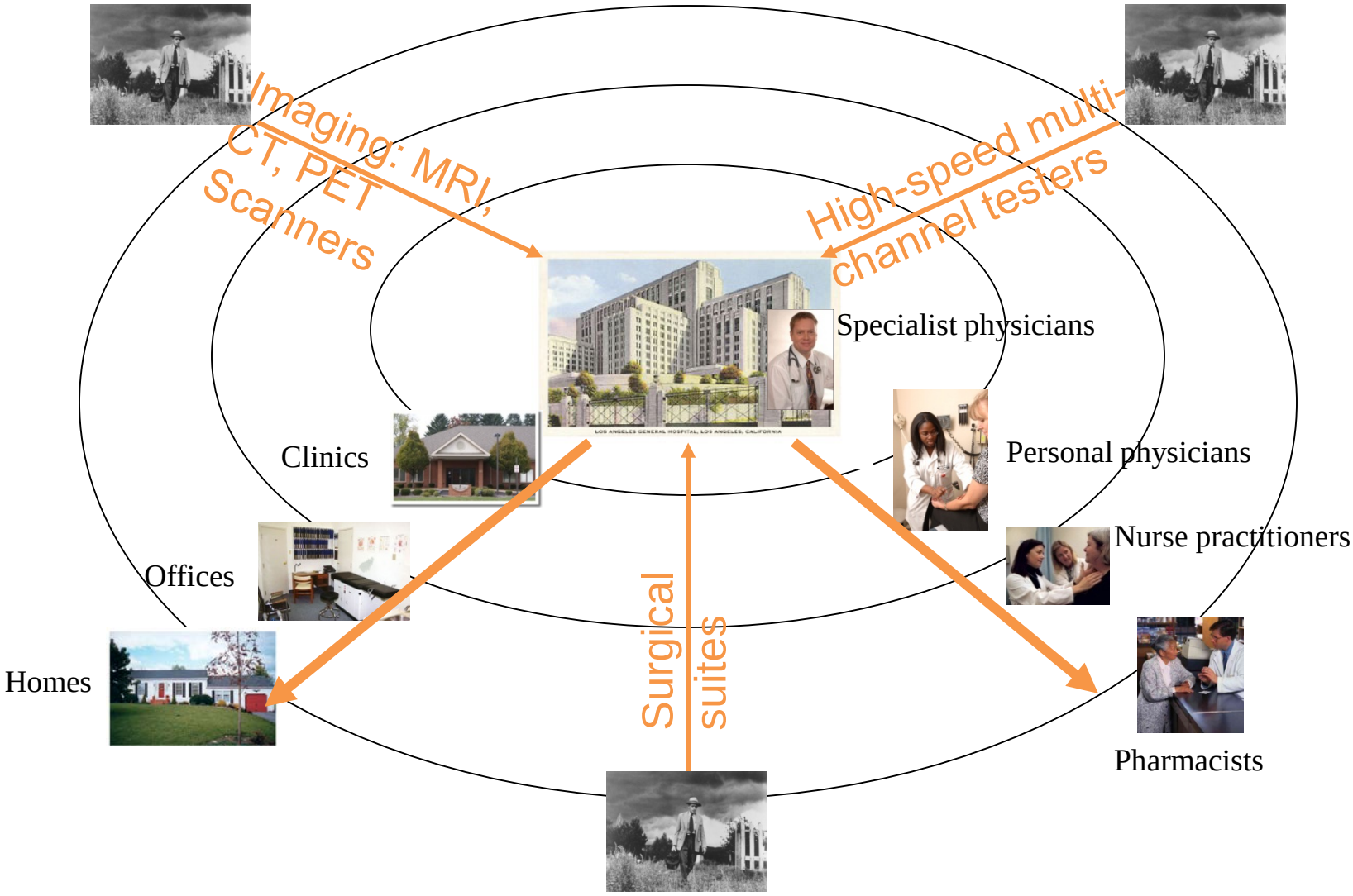
THE WASHINGTON POST

Markets and Technology Innovations



Adapted from *The Innovator's Dilemma*, Clayton Christensen

The decentralization that follows centralization is only beginning in healthcare



UMBC Bio-MOD Platform

Developed from DARPA funding, part of DARPA's Battlefield Medicine Program

Compact and robust system, designed for the automated manufacture of biologics at the point-of-care.

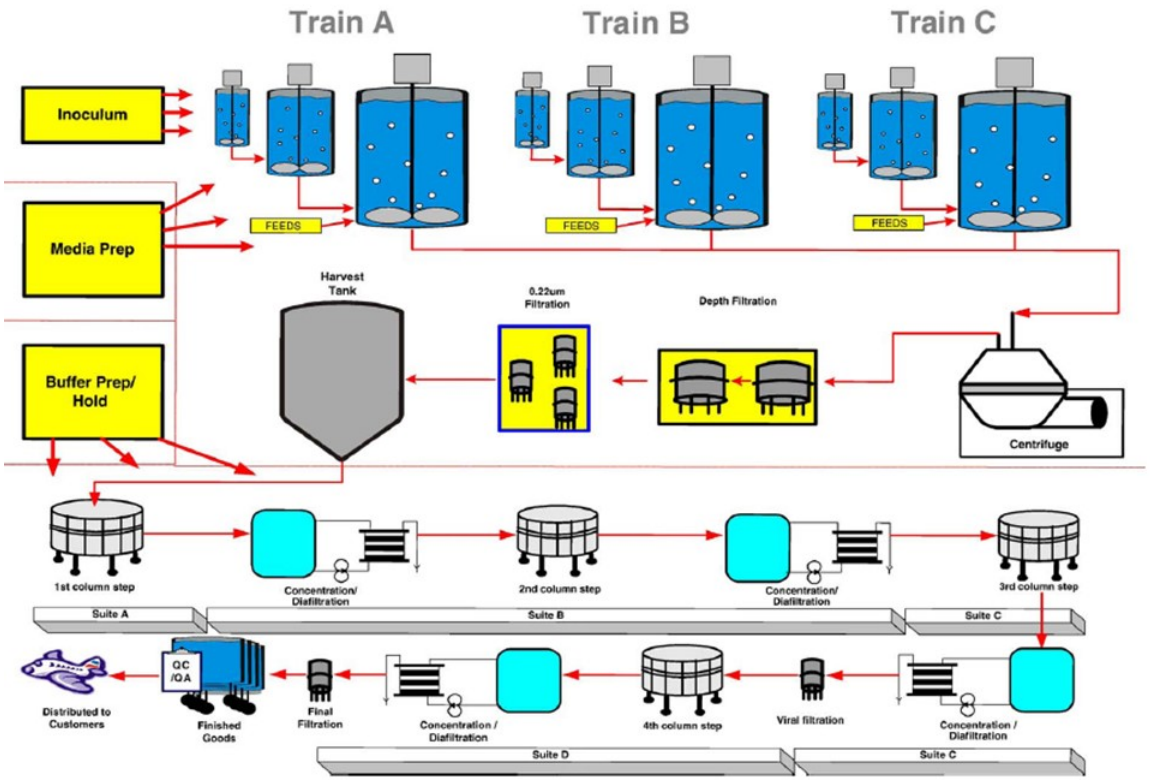
Includes end-to-end manufacturing chain (including downstream processing) in continuous flow within a microfluidics-based platform

- built in expression, purification and onboard quality control capabilities.

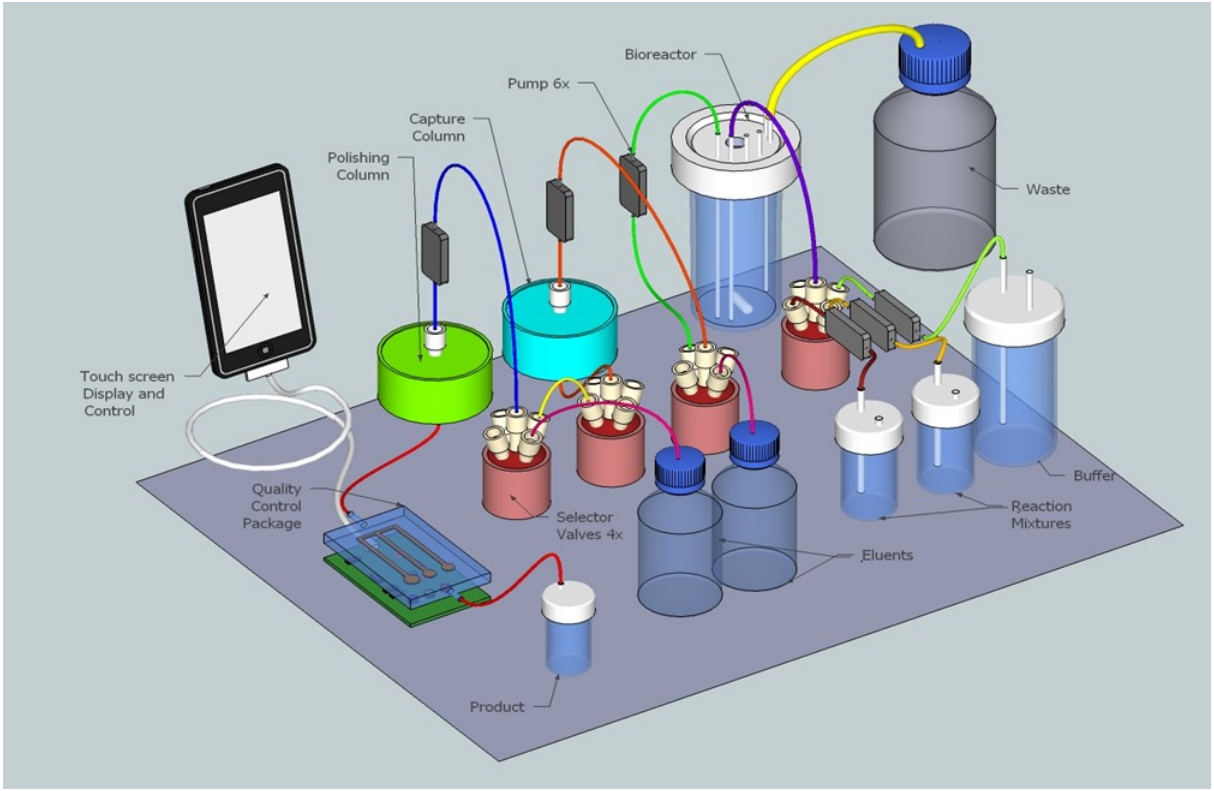
Designed for operational rigor and reproducible protein production within a few hours.

Successfully demonstrated POC with several target proteins, including relevant molecules of interest to the military and biodefense communities.

GOAL: Replace this…



Modular, Disposable, GMP Capable Biologics *on Demand*

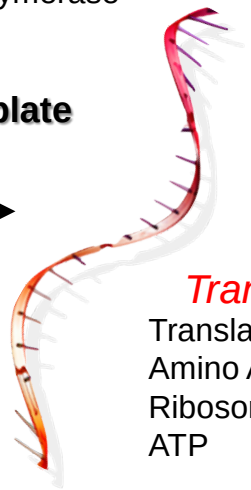


Rapid Expression via *in vitro* Translation (IVT)

CHO Cells

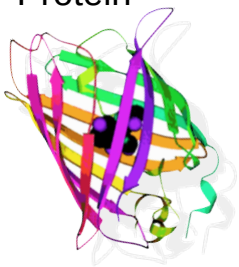
T7 RNA Polymerase
Nucleotides
ATP
DNA Template

RNA



Translation
Translation Factors
Amino Acids
Ribosomes
ATP

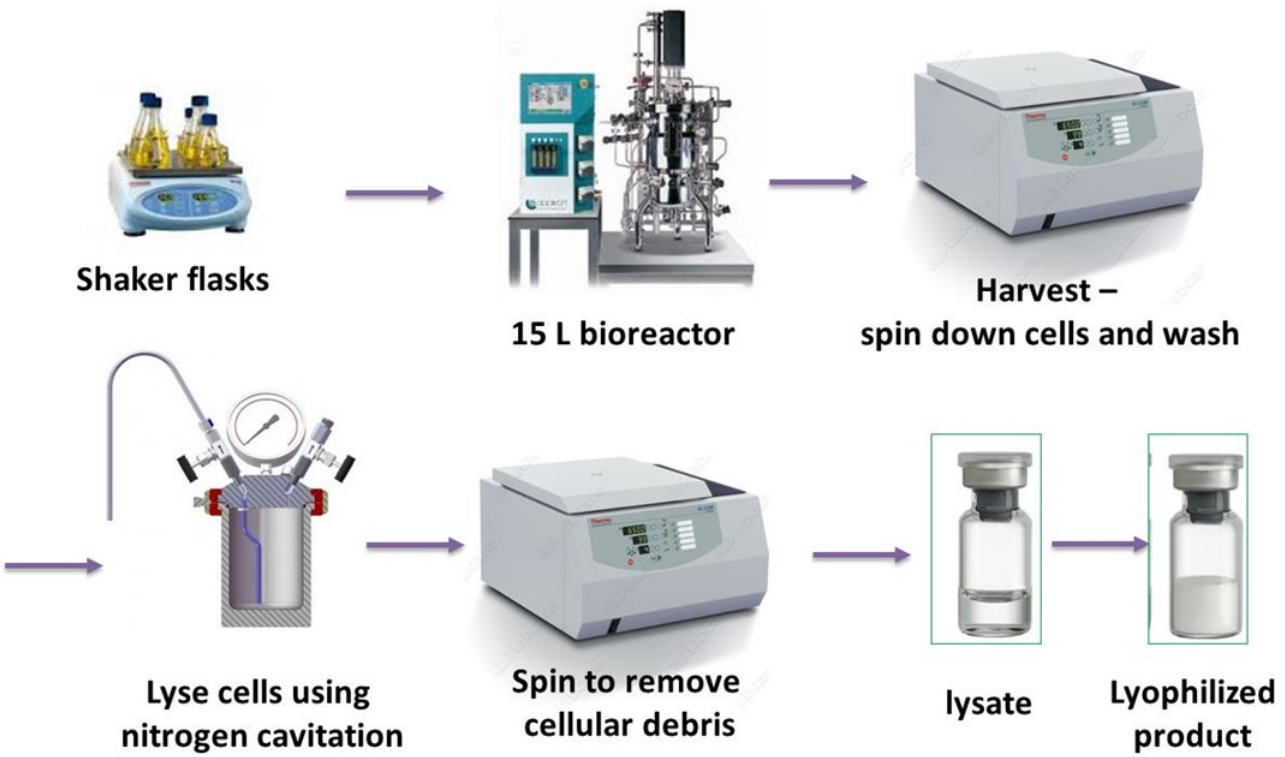
Protein



- 15.0L+ Bioreactor Batch Culture
- Lysis (Cavitation)
- Differential centrifugation to isolate critical organelles
- Lyophilized

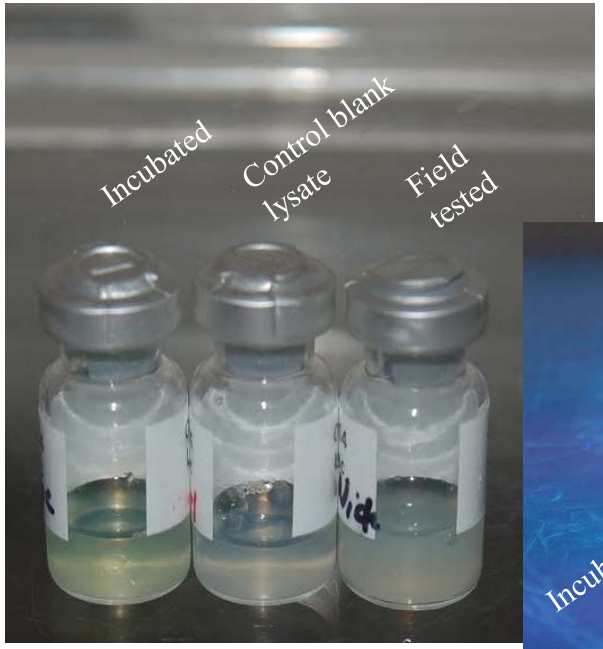
- Advantages of *in vitro* translation (IVT):
  - No cell line development
  - No seed train, passaging, or transfection
  - All live culture scale-up done off line in **generic** single batches

CHO cell-free Lysate Manufacturing Process



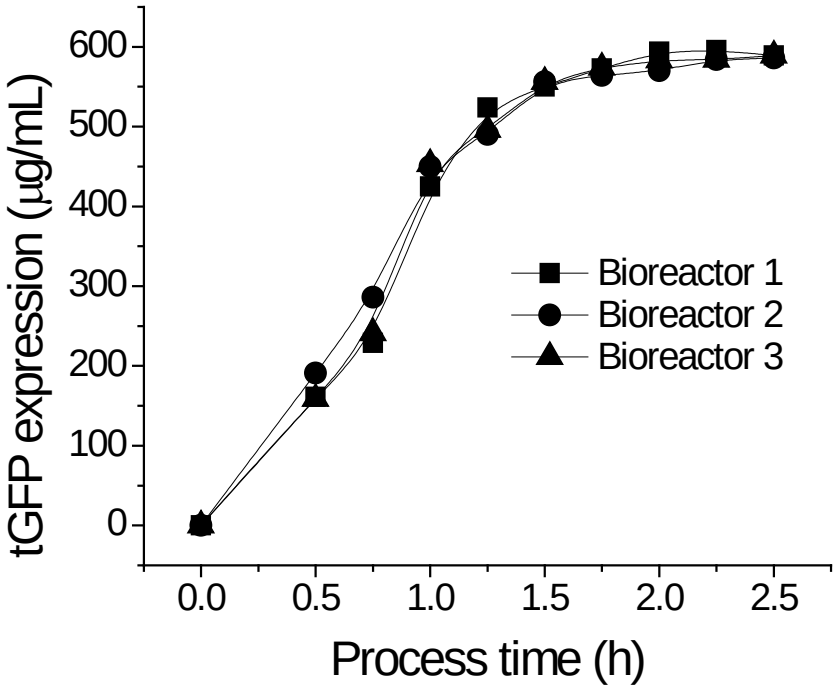
Lyophilized and reconstituted lysate, CHO GFP expression

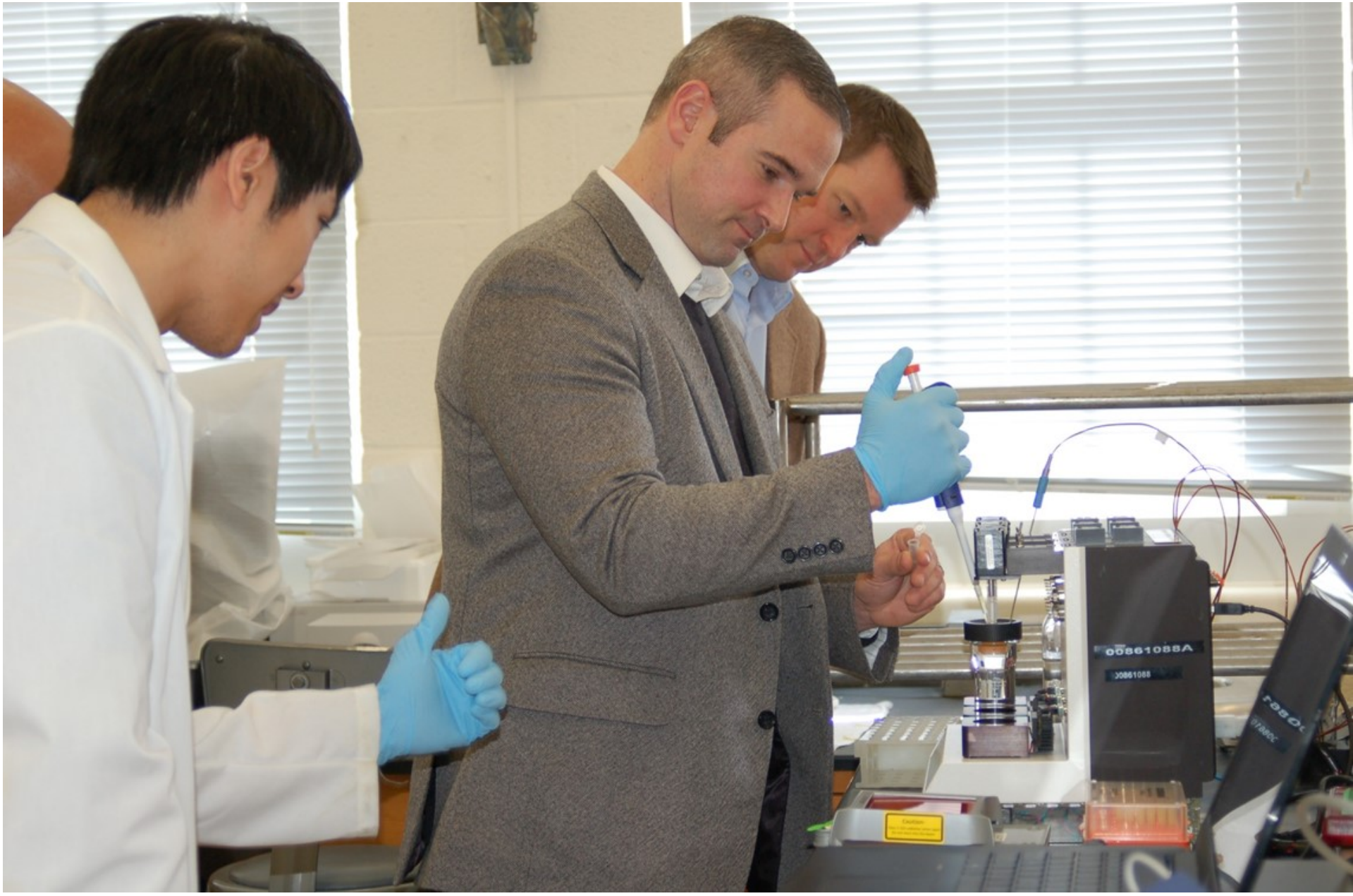
Under normal light



Under UV light

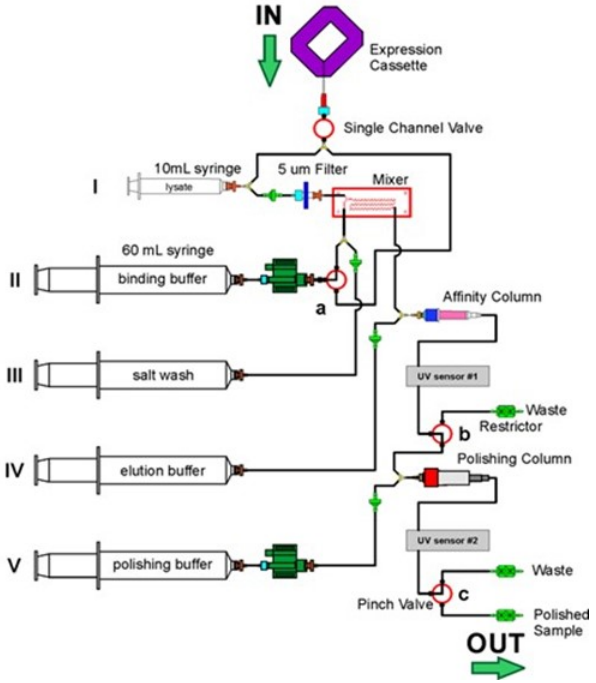
Experiment 192k14




00861088A

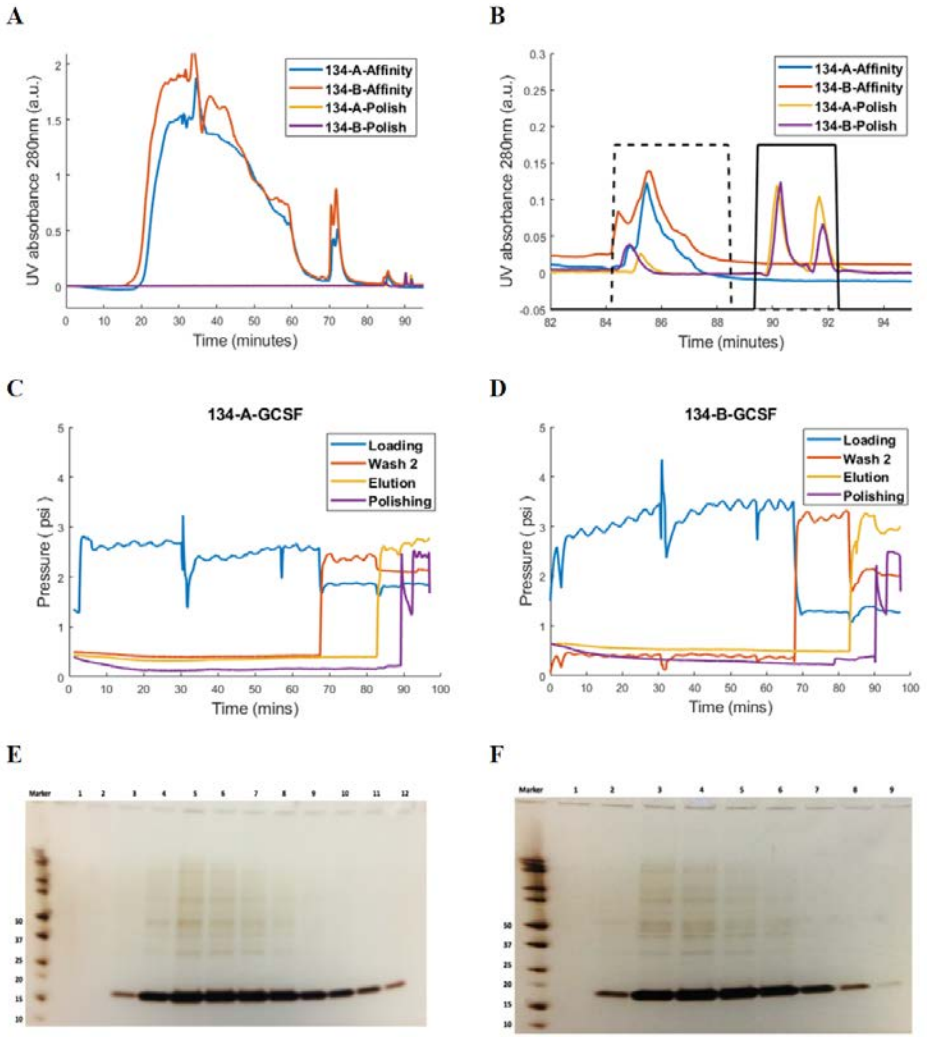
2nd Generation

- Adds polishing step
- Second UV detector

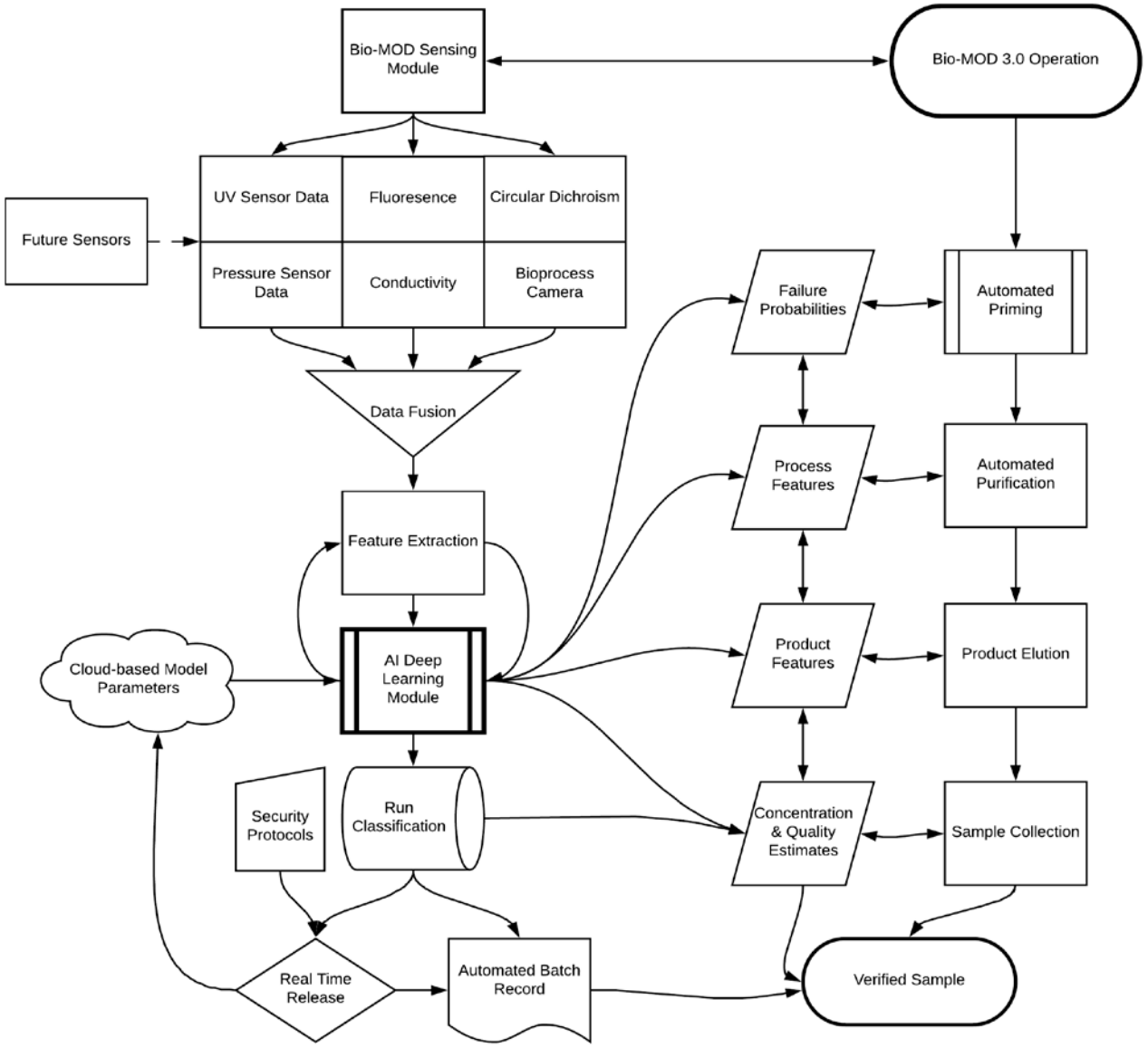



Open Bio-MOD 3.0 Software
Configuration File?
Yes
No
Previous Run Configuration
Default Configuration
Choose Auto-Priming Script
Polishing?
Yes
No
Dual Column Bioprocess Priming
Single Column Bioprocess Priming
Leak or Clog detected?
Yes
No
Bioprocess Troubleshooting
Bubble Detected?
Yes
No
Additional Priming
Interactive Column Insertion
Yes
> 10 p.s.i.?
No
Column Pre-saturation
UV and Pressure Baseline Calculation
Choose Process Script
Harvest Size?
1 mL
2 mL
Load 1mL Process Script
Load 2mL Process Script
Start Purification
> 10 p.s.i.
Yes
No
Process Paused
Leak or Clog Detected?
Yes
No
Process Finished
Retreive Sample
PAT results

Real-time UV (280 nm)and pressure traces of the affinity column purification of various His-tagged proteins



Towards 4.0 Automation and RT Release

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BIOLOGICAL
ON DE
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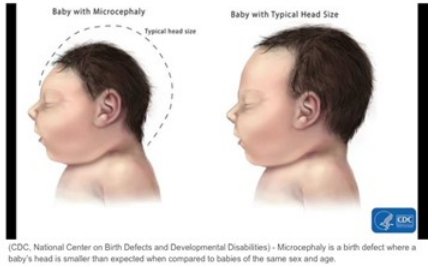
Emerging Infectious Diseases - Zika

Mosquito born Zika outbreak is rapidly spreading in Americas…



(CDC, National Center on Birth Defects and Developmental Disabilities) - Microcephaly is a birth defect where a baby's head is smaller than expected when compared to babies of the same sex and age.

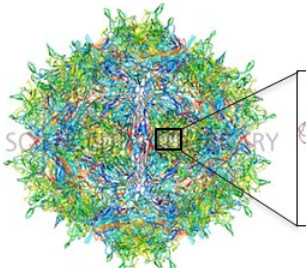
A quote from news:

" The Centers for Disease Control in Atlanta, Georgia, warned last week there now are 107 travel-related cases of the Zika virus in 24 states and the District of Columbia.

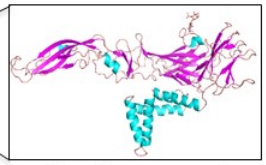
It issued a travel advisory for pregnant women and others who are planning to attend the 2016 Summer Olympic Games in Rio de Janeiro, Brazil, from Aug. 5 to 21."

I. Synthesized the DNA for Zika envelope protein

Time line:

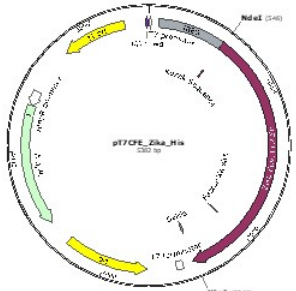


Zika virus



Envelope protein

rDNA synthesized & cloned



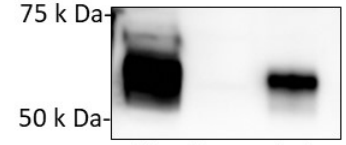
Zika Env. (IVT Plasmid)

about 2 weeks

CHO lysate (Lyophilized)

In vitro coupled reaction

Expression in cassette

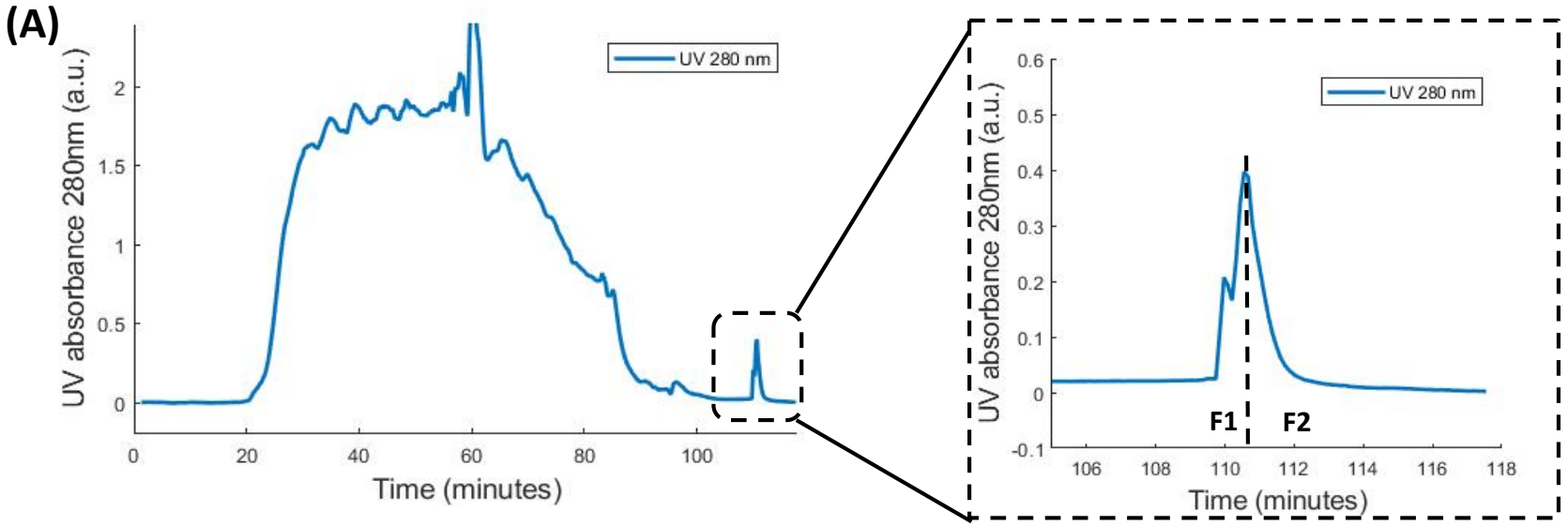


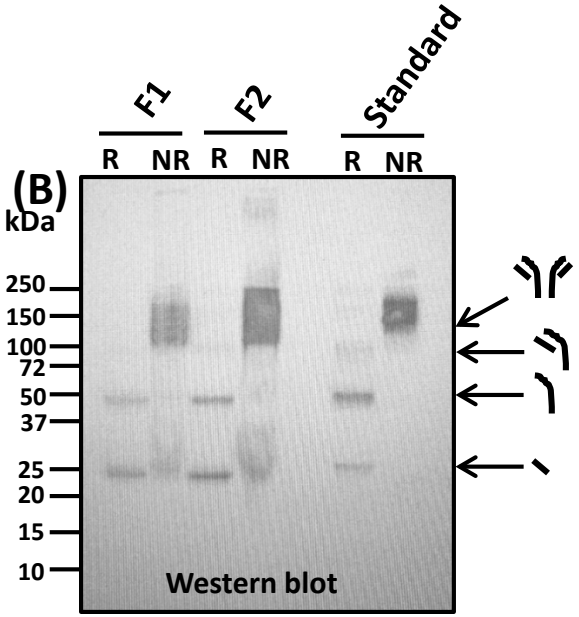
Zika Env. protein

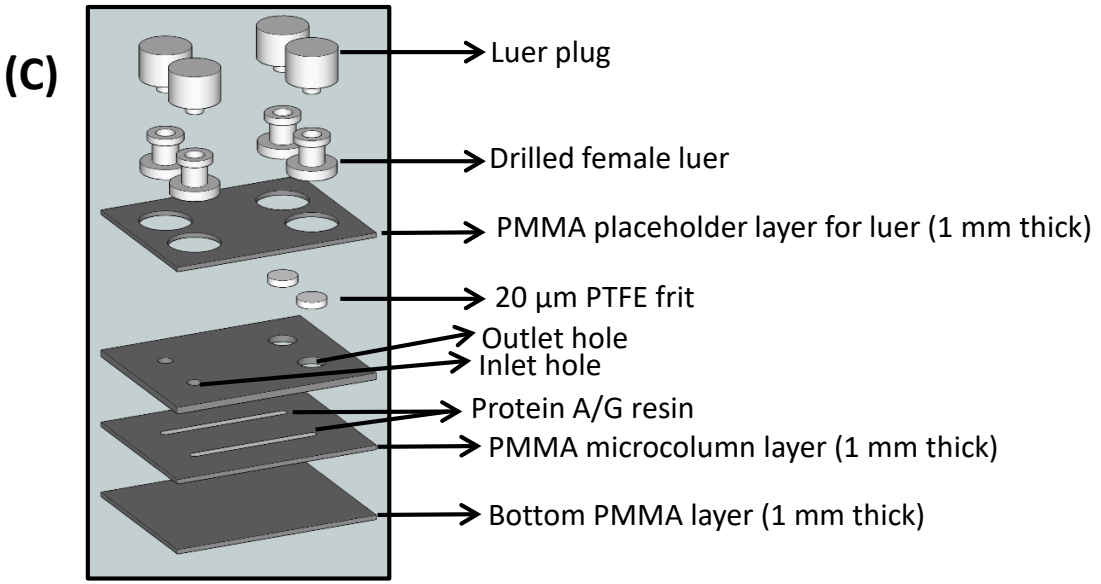
< 24 h

II. Expression & purification

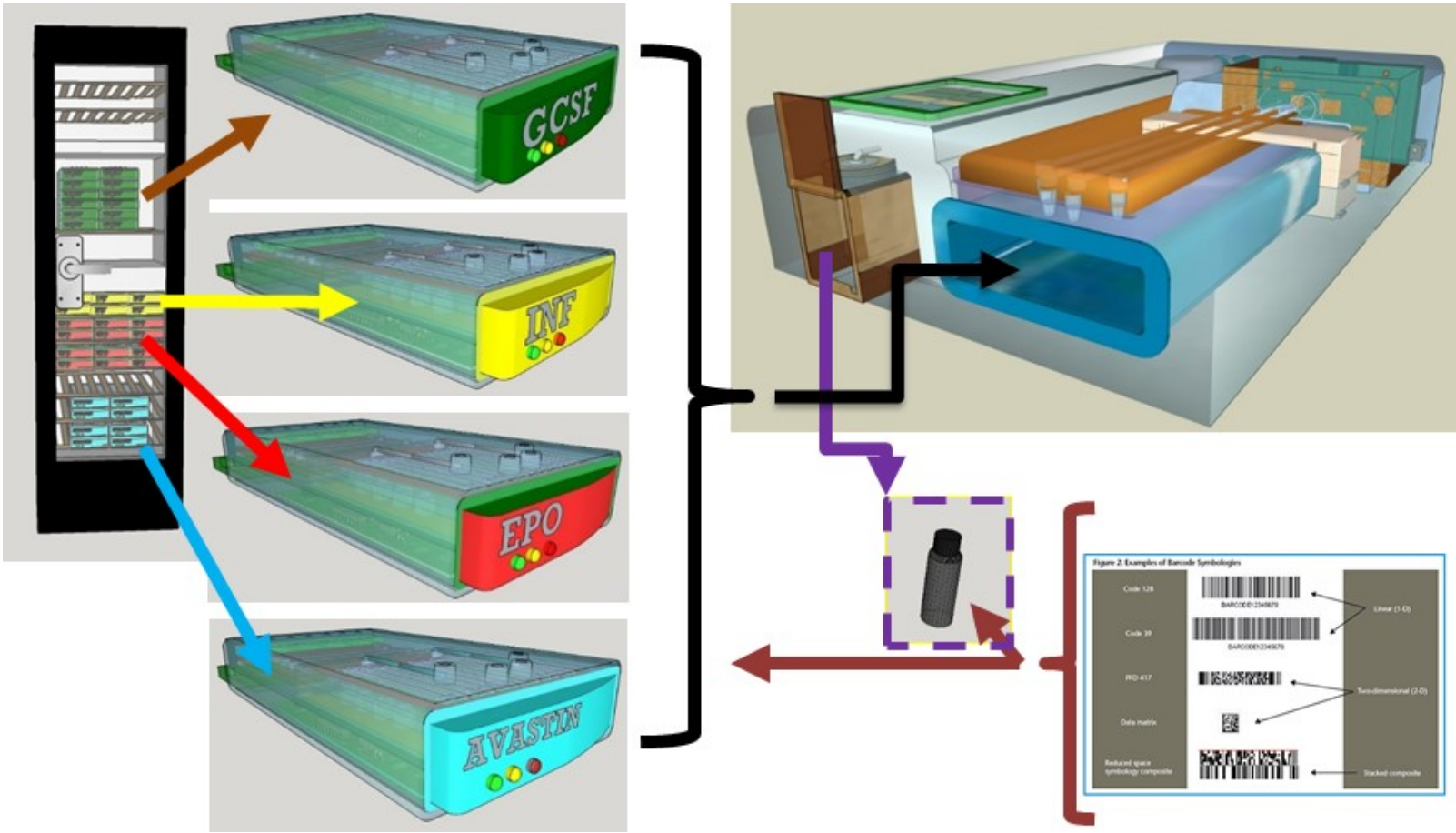
Figure 5: Bio-MOD Purification of Humira mAb using Protein A/G µcolumn







Concept for Facility of the Future: JHU Focus Group Led Design



nature biomedical engineering

ARTICLES

https://doi.org/10.1038/s41551-018-0259-1

Point-of-care production of therapeutic proteins of good-manufacturing-practice quality

Rajani Adiga[1], Mustafa Al-adhami[1,2], Abhay Andar[1], Shayan Borhani[1,3], Sheniqua Brown[1,3], David Burgenson[1,3], Merideth A. Cooper[4], Sevda Deldari[1,3], Douglas D. Frey[1,3], Xudong Ge[1,3], Hui Guo[1,3], Chandrasekhar Gurramkonda[1], Penny Jensen[5], Yordan Kostov[1,3], William LaCourse[6], Yang Liu[3], Antonio Moreira[1,3], KarunaSri Mupparapu[1], Chariz Peñalber-Johnstone[1], Manohar Pilli[1], Benjamin Punshon-Smith[7], Aniruddha Rao[1,6], Govind Rao[1,3*], Priyanka Rauniyar[8], Sergei Snovida[5], Kanika Taurani[1], Dagmawi Tilahun[1], Leah Tolosa[1,3], Michael Tolosa[1], Kevin Tran[1], Krishna Vattem[5], Sudha Veeraraghavan[9,10], Brandon Wagner[1], Joshua Wilhide[8], David W. Wood[4] and Adil Zuber[3]

https://rdcu.be/2Jk8

November 12, 2018 and 2019

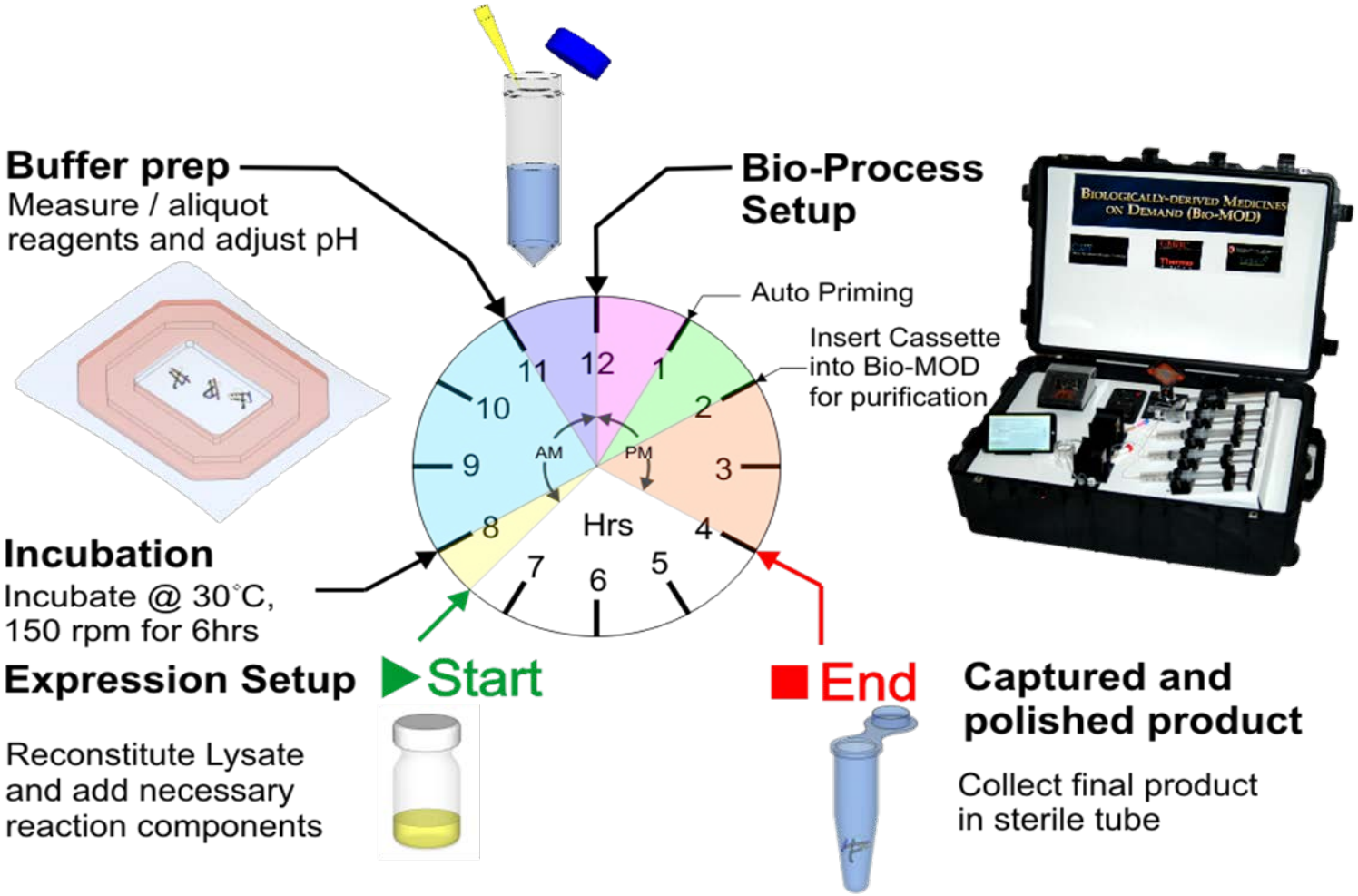
FDA Approves Radiation Medical Countermeasure

Acute Radiation Syndrome | Animal Rule Approval | Radiation Emergency Preparedness | Contacts

FDA approves Neupogen for treatment of patients with radiation-induced myelosuppression following a radiological/nuclear incident

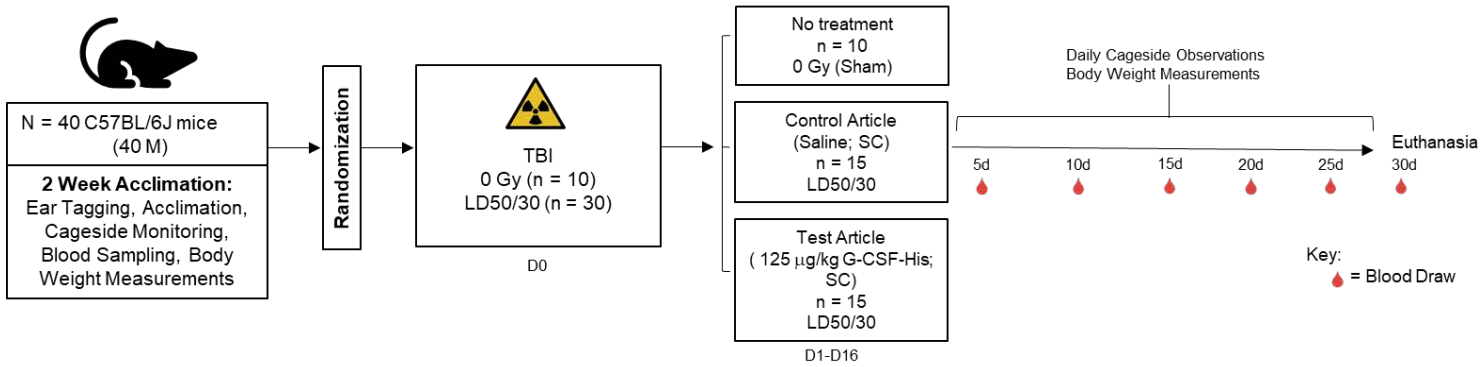
On March 30, 2015, FDA approved use of Neupogen (filgrastim) to treat adult and

Daily Manufacturing Schedule



Bio-MOD Process Clock

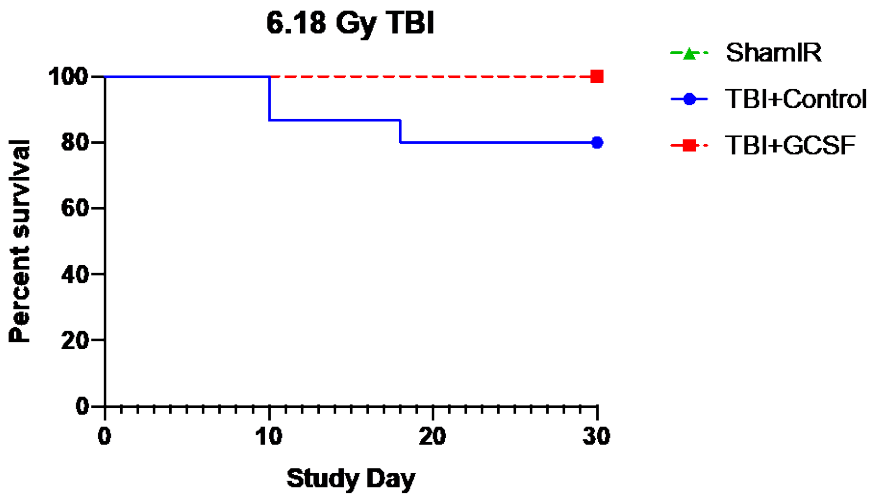
Study Design and Timeline



The study will enroll up to 40 animals exposed to sham or total body irradiation (TBI). Blood will be collected at baseline and at scheduled timepoints post-irradiation. Animals receiving test or control article will be treated beginning 24 ± 2 hours post-irradiation (D1) and treatment will continue daily until D16. Animals will be monitored for 30 days post-exposure.

Study Initiation	October 24, 2018
Receipt of Animals	November 14, 2018
Allocation of Animals	November 13, 2018
Irradiation (D0)	November 29, 2018
Treatment Initiation	November 30, 2018
Dosing Completion	December 15, 2018
Experimental (In-life) End	December 29, 2018

Kaplan-Meier curves for 30-day survival



Treatment (Tx) Group	Radiation Exposure	Radiation Dose (Gy)	Group Size	Number of Survivors at D30
No Tx	Sham	0	10	10
Saline	TBI	6.18	15	12
G-CSF-His	TBI	6.18	15	15

Received: 24 October 2019 | Revised: 13 January 2020 | Accepted: 20 January 2020

DOI: 10.1002/btpr.2970

BIOTECHNOLOGY PROGRESS

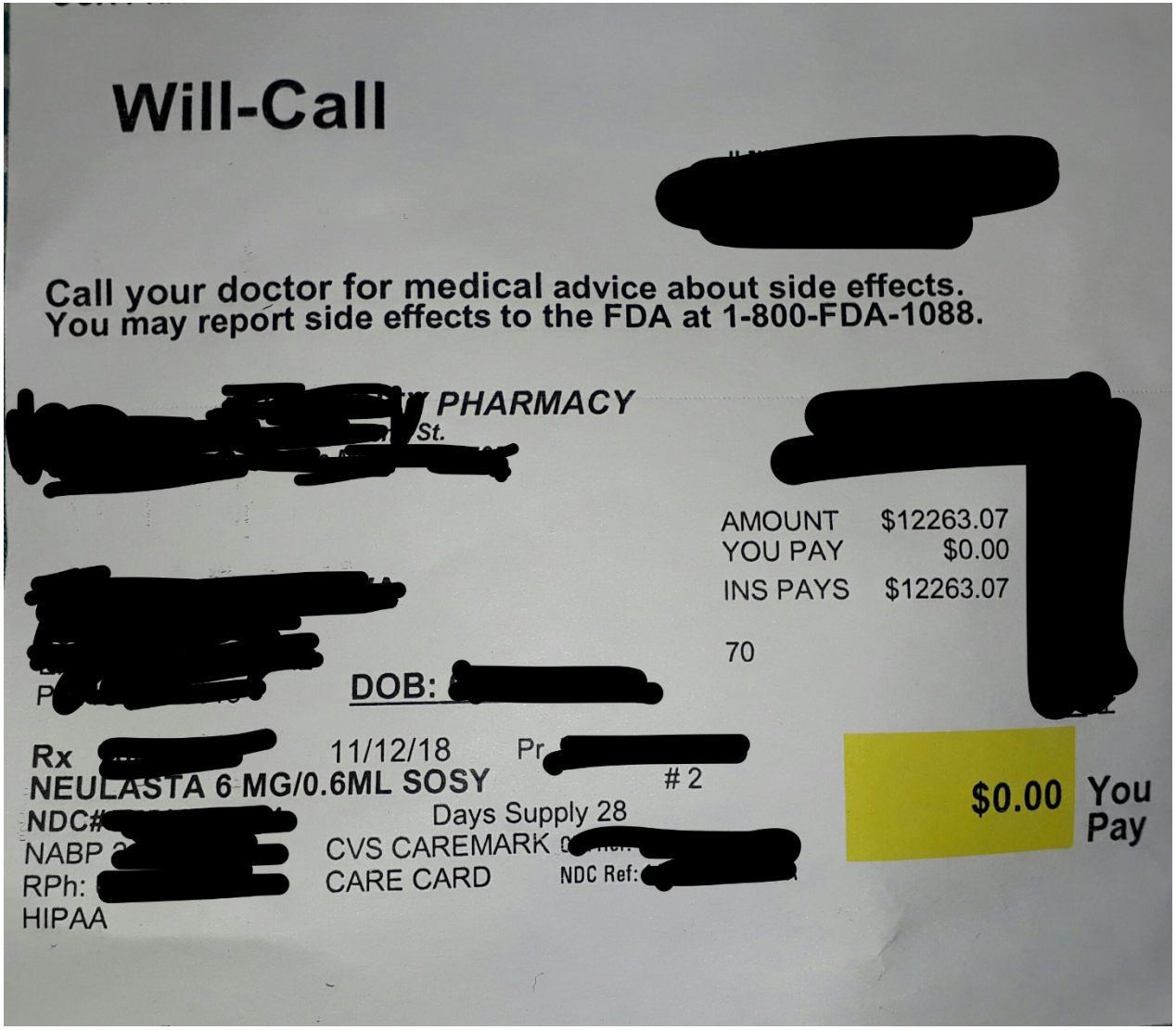
RESEARCH ARTICLE

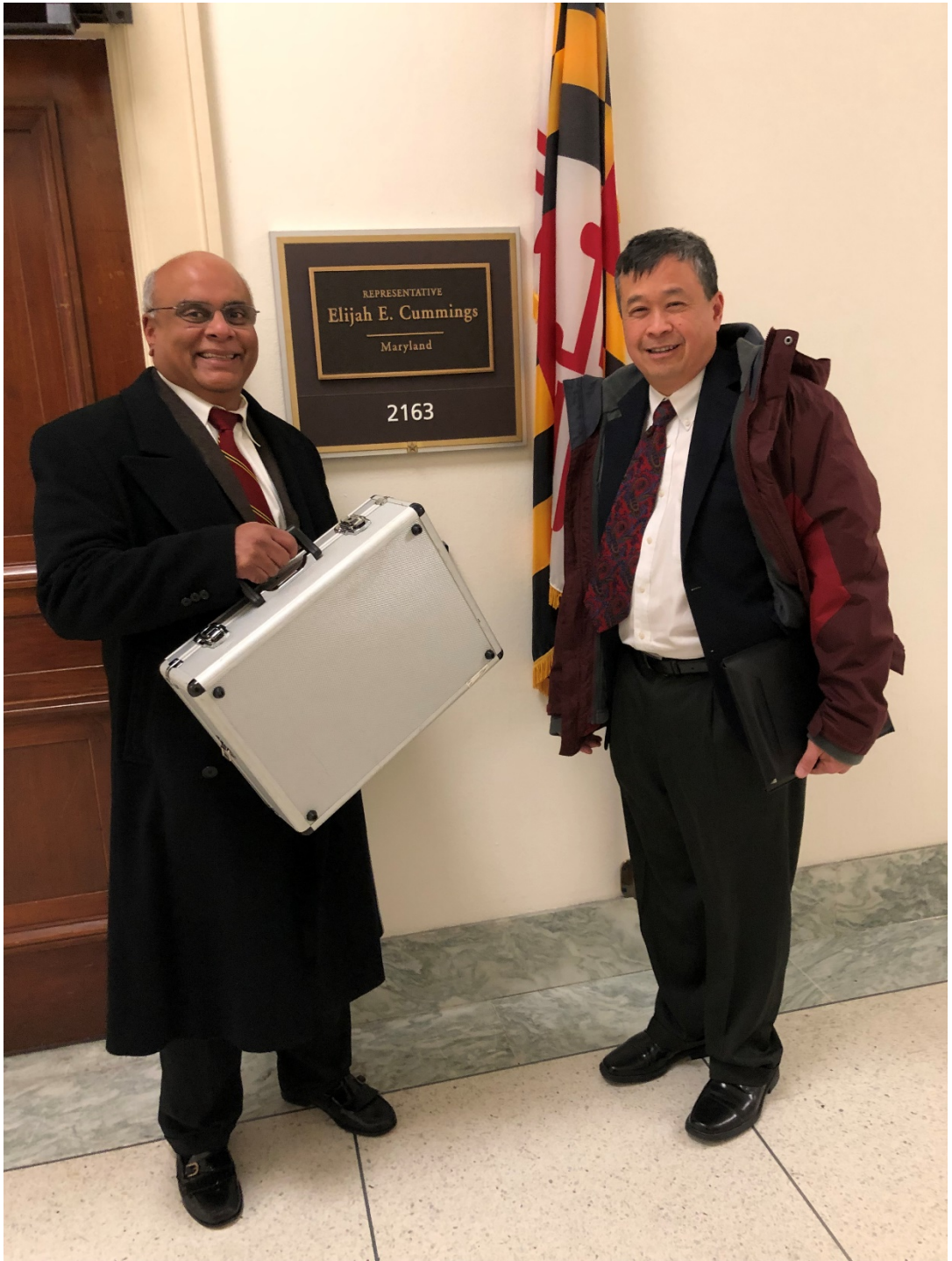
Bioseparations and downstream processing

Manufacturing biological medicines on demand: Safety and efficacy of granulocyte colony-stimulating factor in a mouse model of total body irradiation

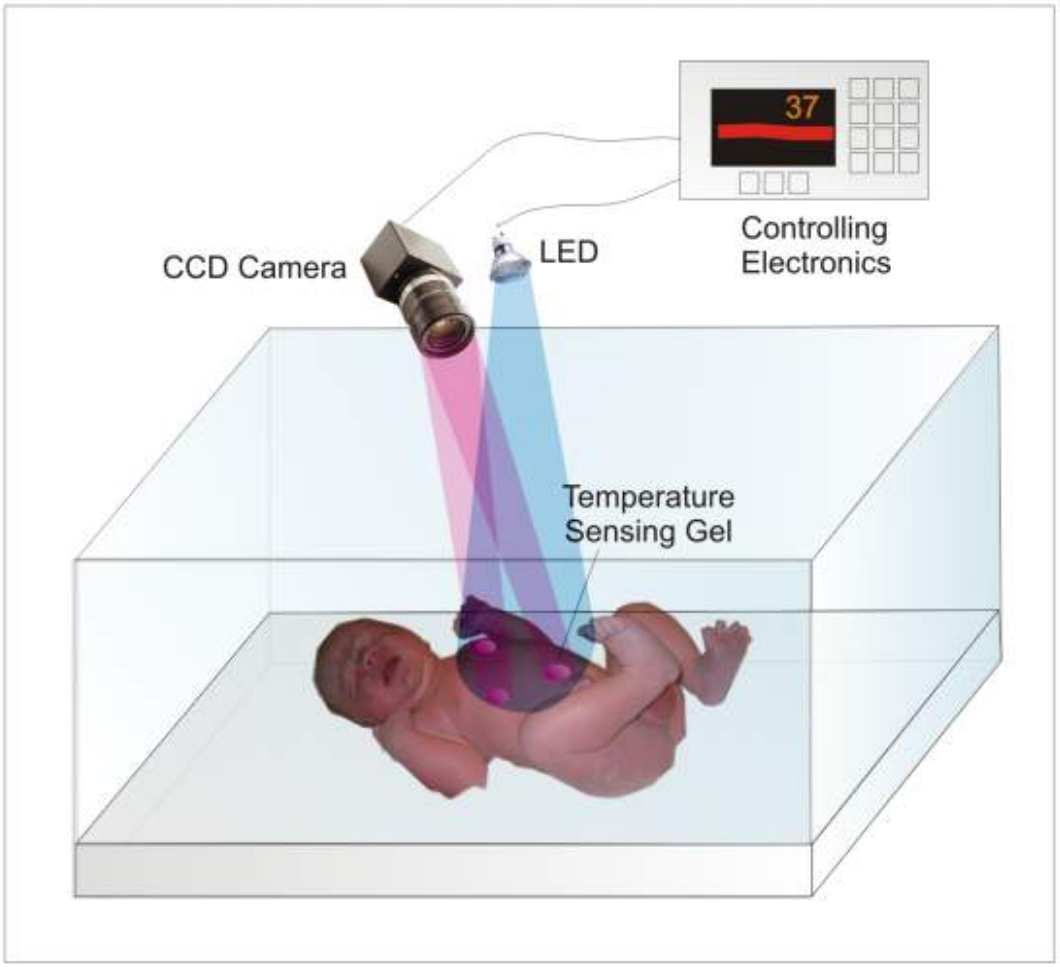
Rajani Adiga[1] | Abhay Andar[1] | Shayan Borhani[1] | David Burgenson[1] | Sevda Deldari[1] | Douglas Frey[1] | Xudong Ge[1] | Chandrasekhar Gurramkonda[1] | Erick Gutierrez[1] | Isabel L. Jackson[2] | Yordan Kostov[1] | Yang Liu[1] | Diana Newman[2] | Joseph Piegols[2] | Benjamin Punshon-Smith[1] | Govind Rao[1] | Leah Tolosa[1] | Mike Tolosa[1] | Zeljko Vujaskovic[2] | Chelsea Wagner[2] | Lynn Wong[1] | Andrew Zodda[2]

This is now personal




REPRESENTATIVE
Elijah E. Cummings
Maryland
2163

GE Healthcare Collaboration



Non-contact or non-invasive parameter sensing.

Brings LIGHT
to
LIFE

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The Eureka Moment!

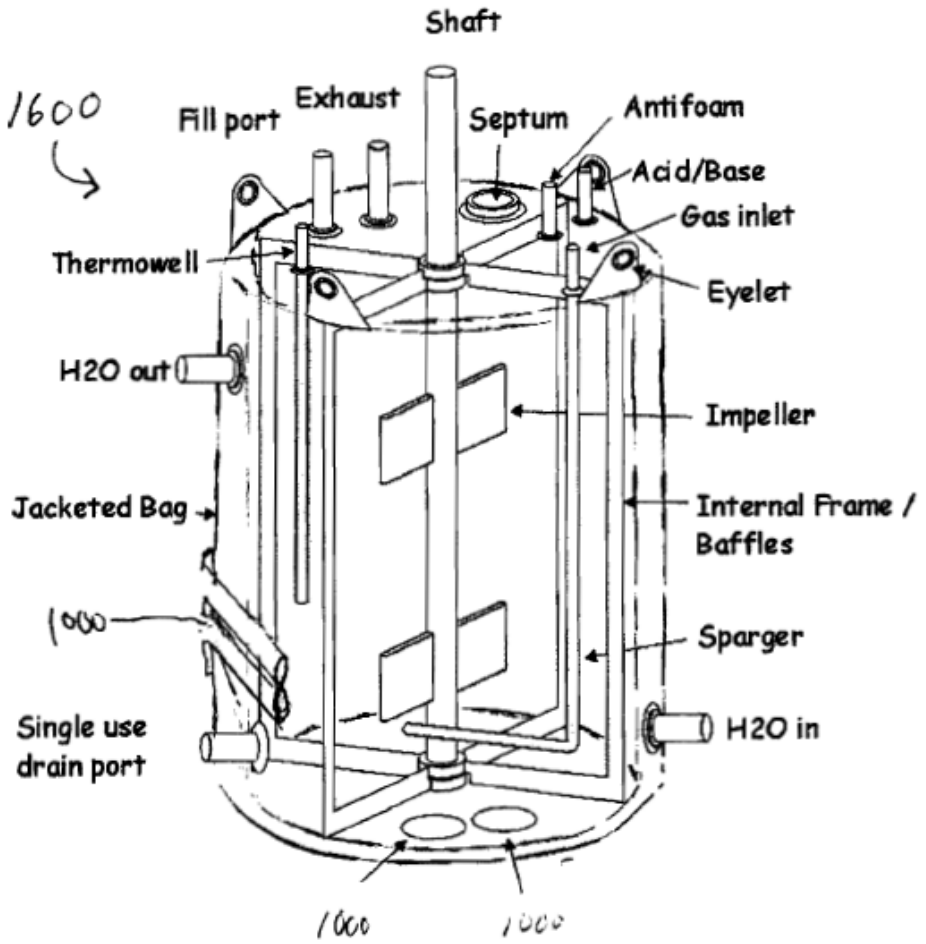
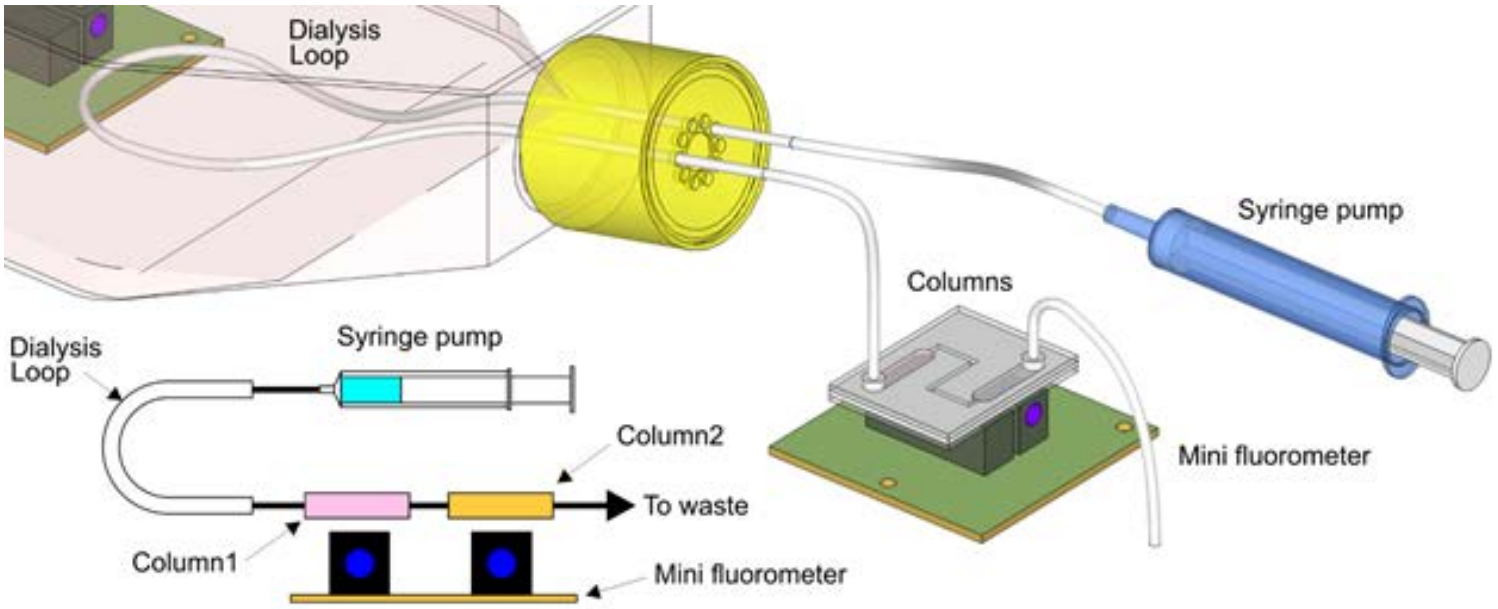
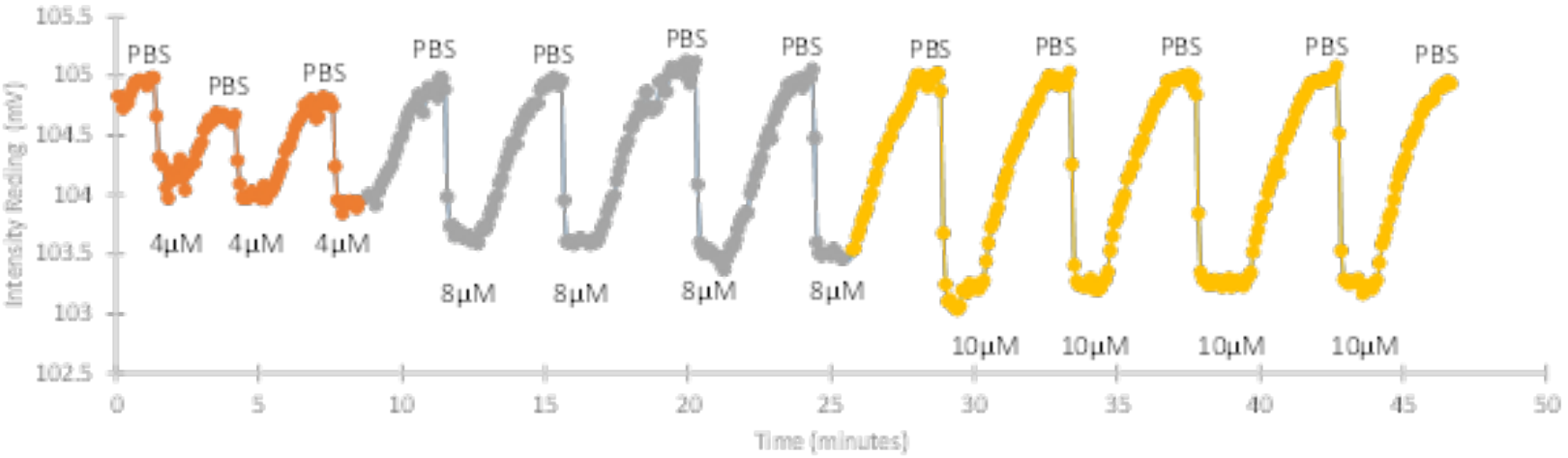
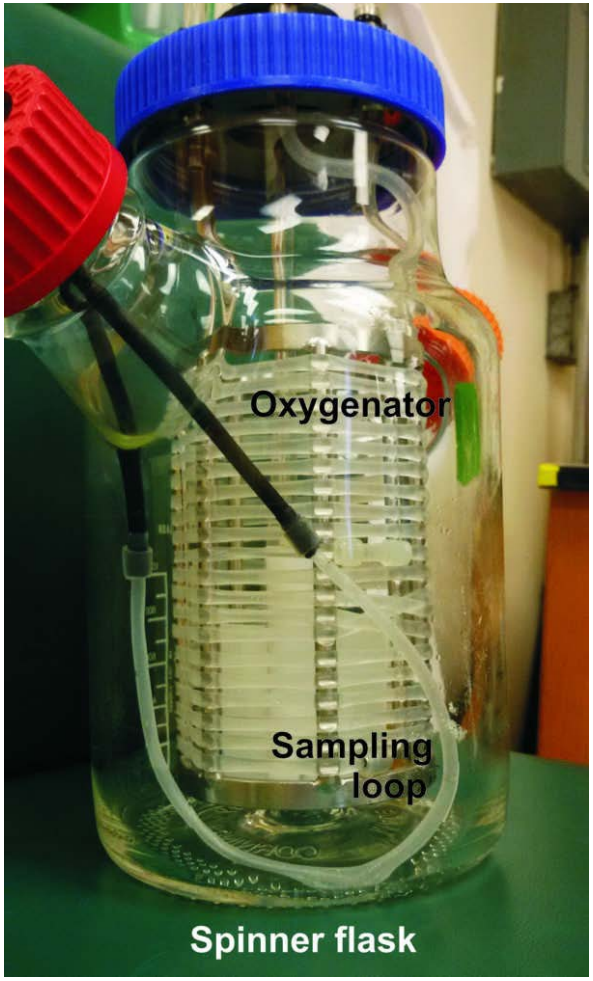


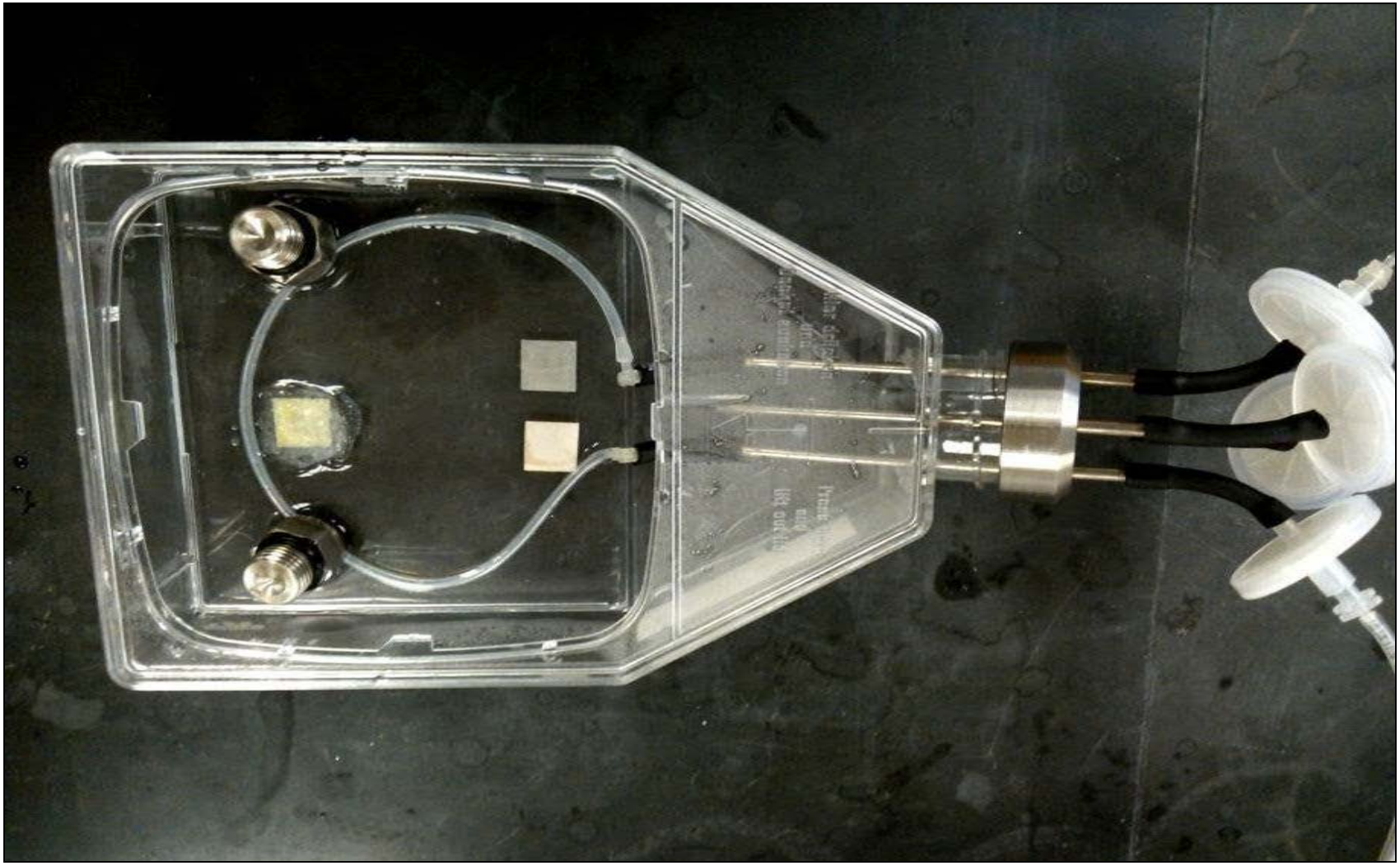
Fig 12

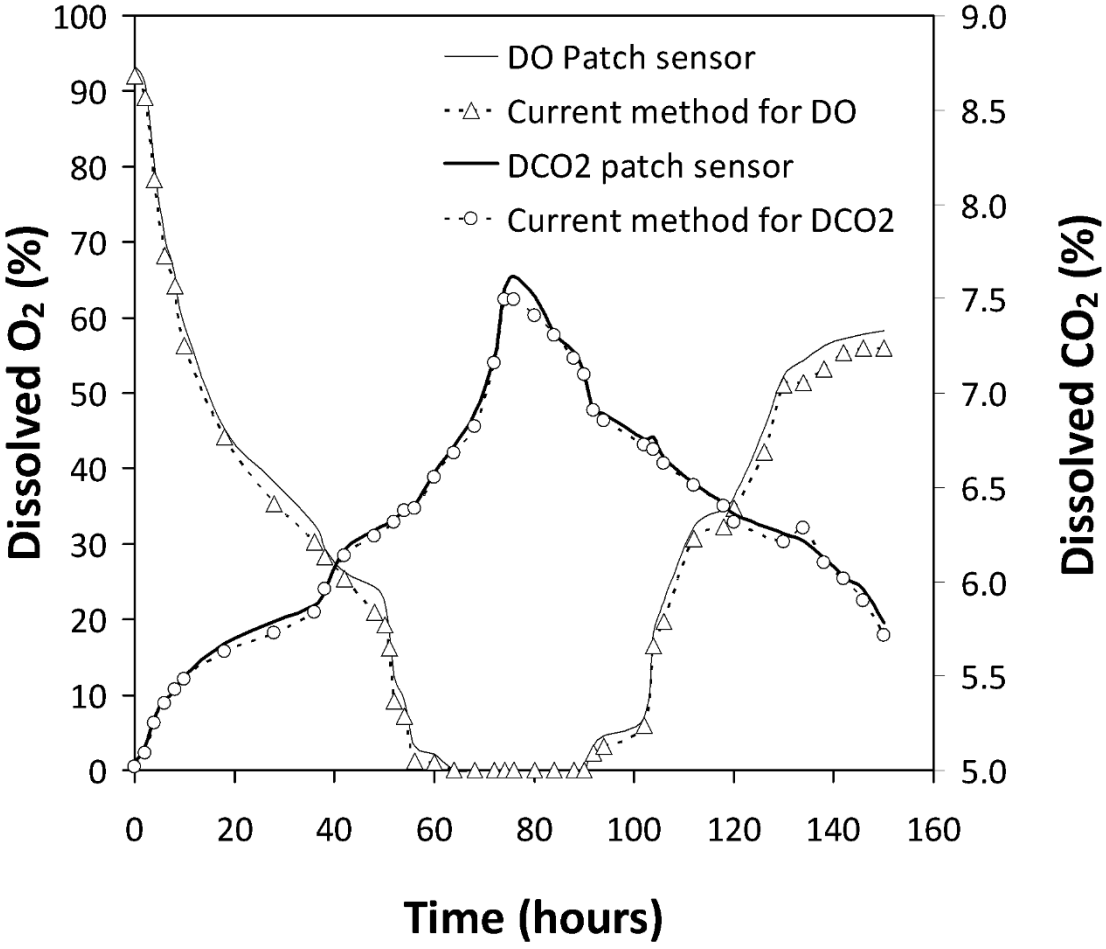

Dialysis Loop
Syringe pump
Columns
Mini fluorometer
Dialysis Loop
Syringe pump
Column2
To waste
Column1
Mini fluorometer


Intensity Reading (mV)
105.5
105
104.5
104
103.5
103
102.5
PBS
4μM
8μM
10μM
0
5
10
15
20
25
30
35
40
45
50
Time (minutes)

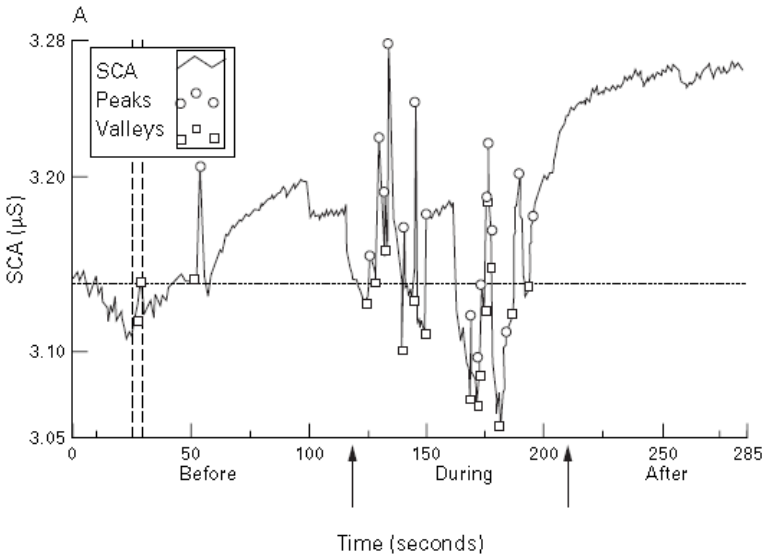

Oxygenator
Sampling loop
Spinner flask

Top view




DO Patch sensor
Current method for DO
DCO2 patch sensor
Current method for DCO2
Dissolved O2 (%)
Dissolved CO2 (%)
Time (hours)
100
90
80
70
60
50
40
30
20
10
0
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
0
20
40
60
80
100
120
140
160

Current practice for blood glucose – collect blood by arterial/venous draw or a heel lance



Arch Dis Child Fetal Neonatal Ed 2000;83:F143–F147

Risks –
- Acquired infections
- Anemia
- May need transfusions

Causes pain and undue distress with short and long term consequences

Transdermal Glucose Monitoring Concept

Figure 1 Noninvasive glucose monitoring system for the neonatal intensive care unit: (left) Sample head for the noninvasive transdermal measurement of glucose on the thigh of the neonate; (right) the complete system showing the instrumentation.

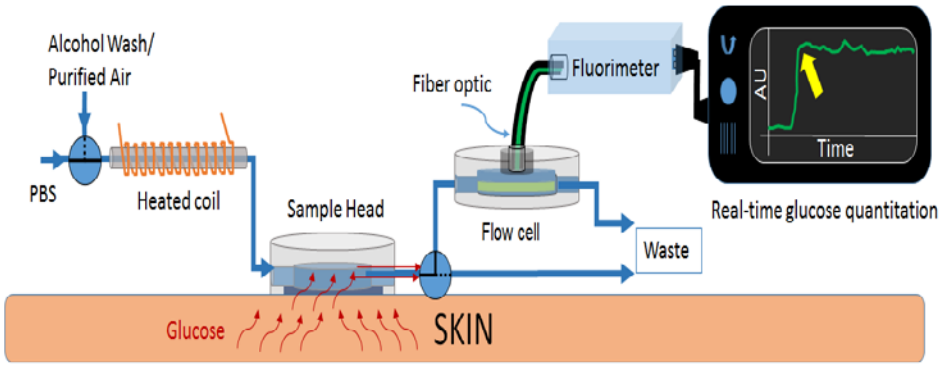
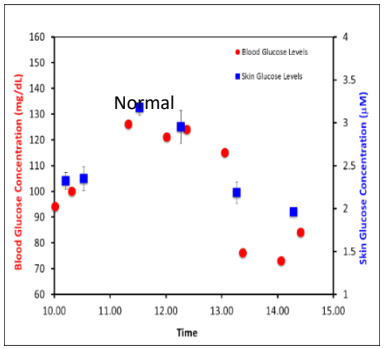
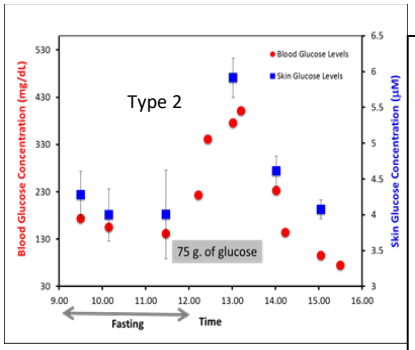


Figure 2 The noninvasive glucose monitoring system for neonates showing the fluidic system with the sample head affixed to the intact skin and the analytics system comprised of the fiber optic biosensor, fluorimeter and read out.

Adult data show good correlation between blood glucose and transdermal glucose





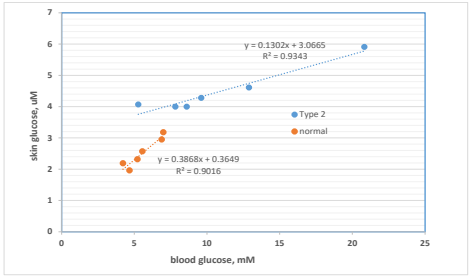
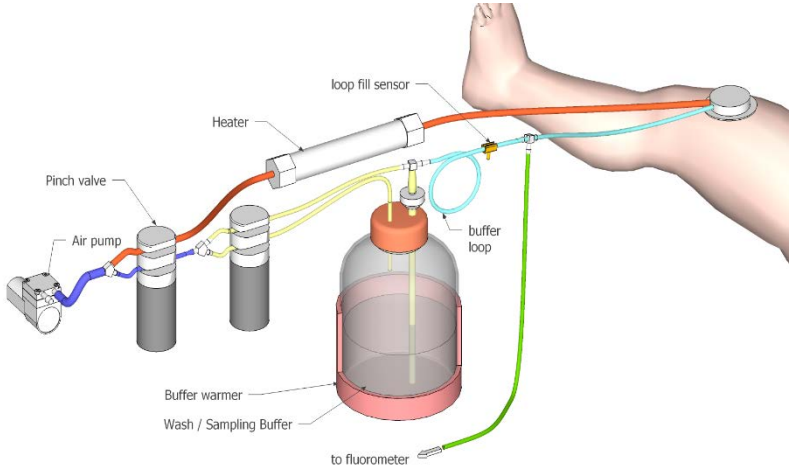
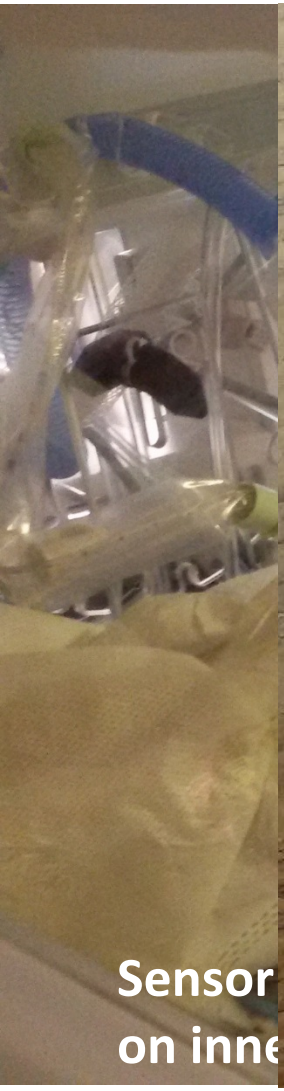


Figure 3 Goal is Lower cost noninvasive glucose sensing system.





Sensor
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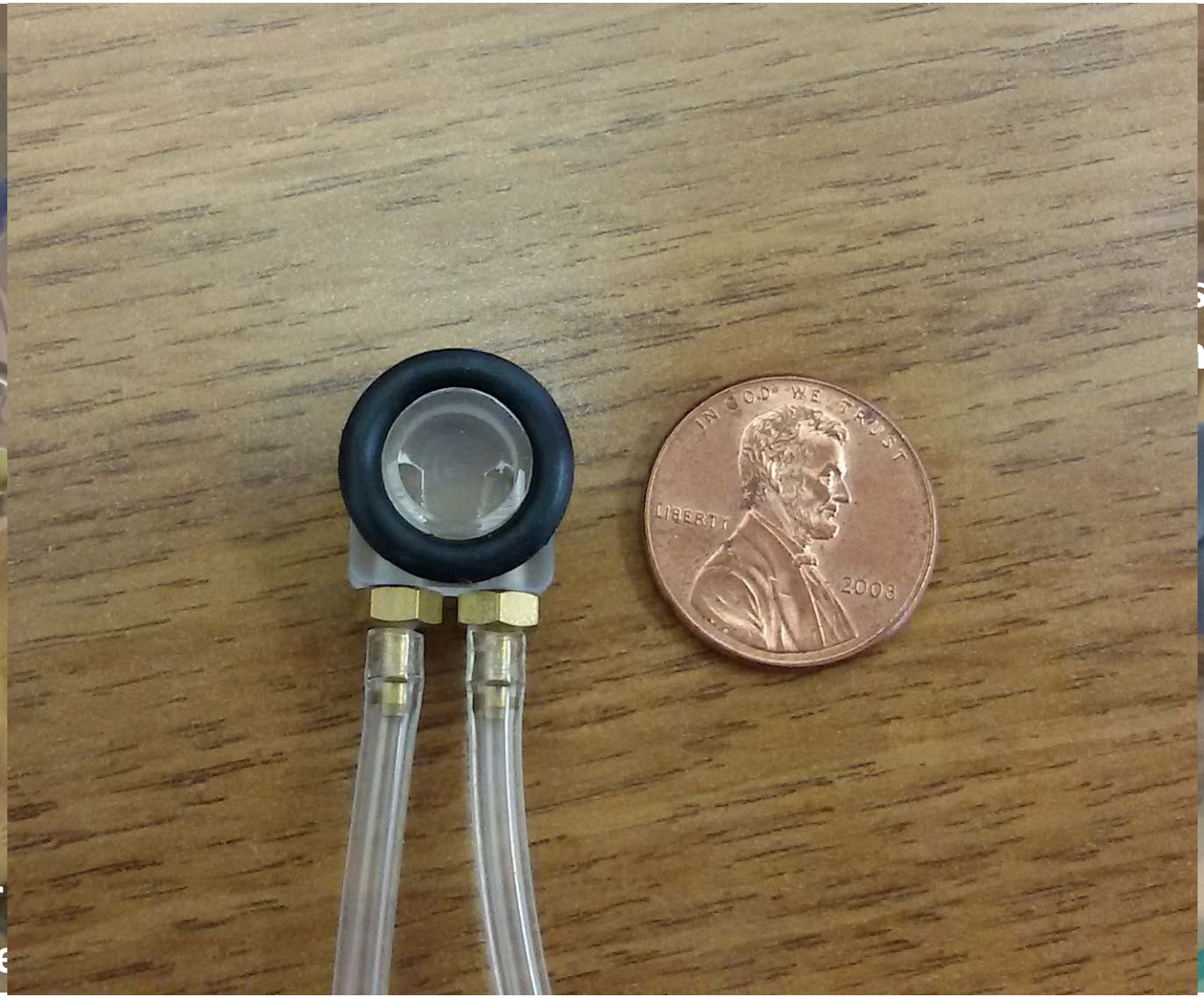

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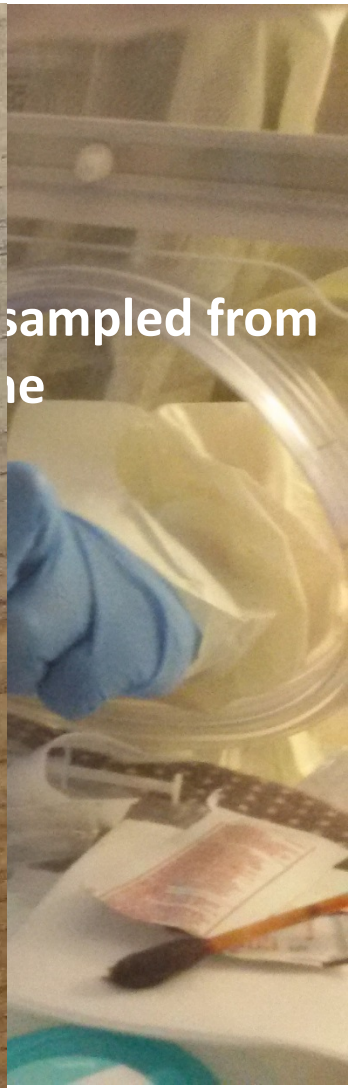
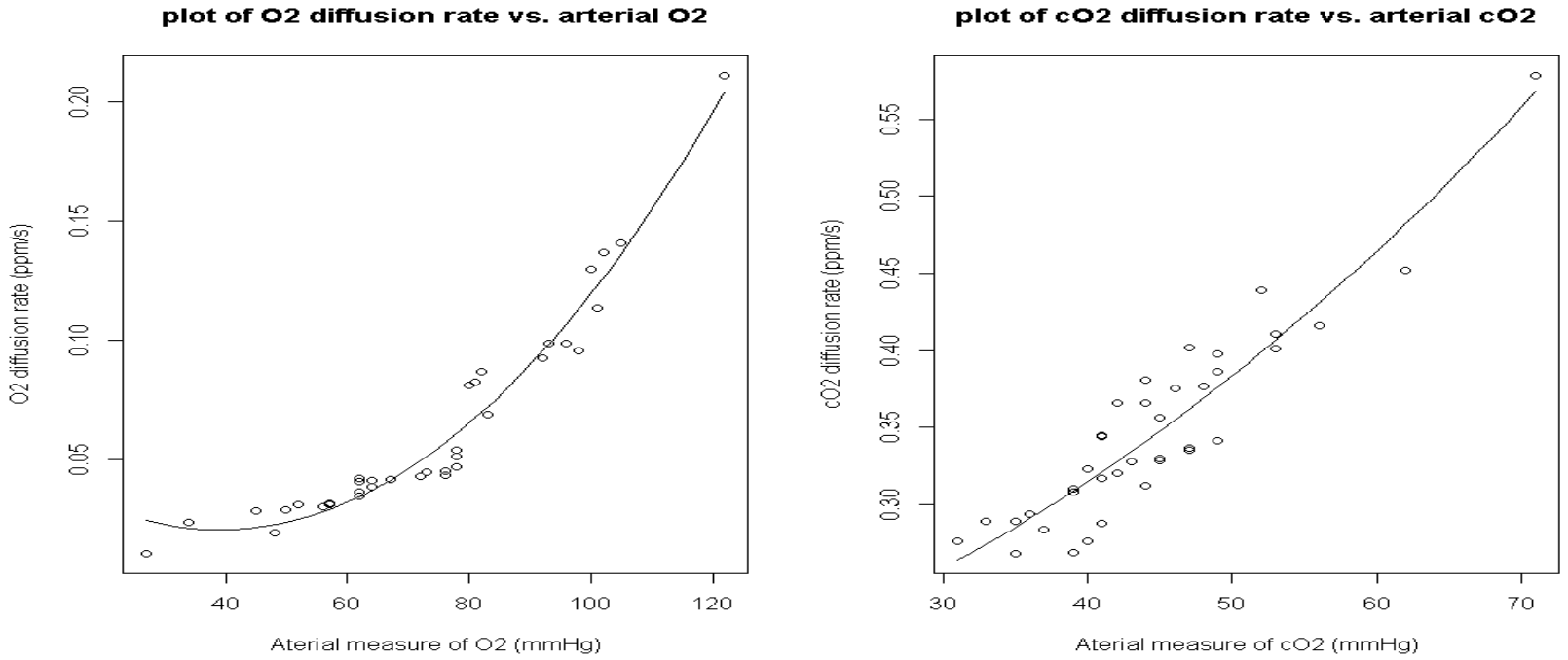


Correlation between the initial transcutaneous diffusion rates and their respective arterial blood partial pressures for 9 neonates with NO prior calibration!



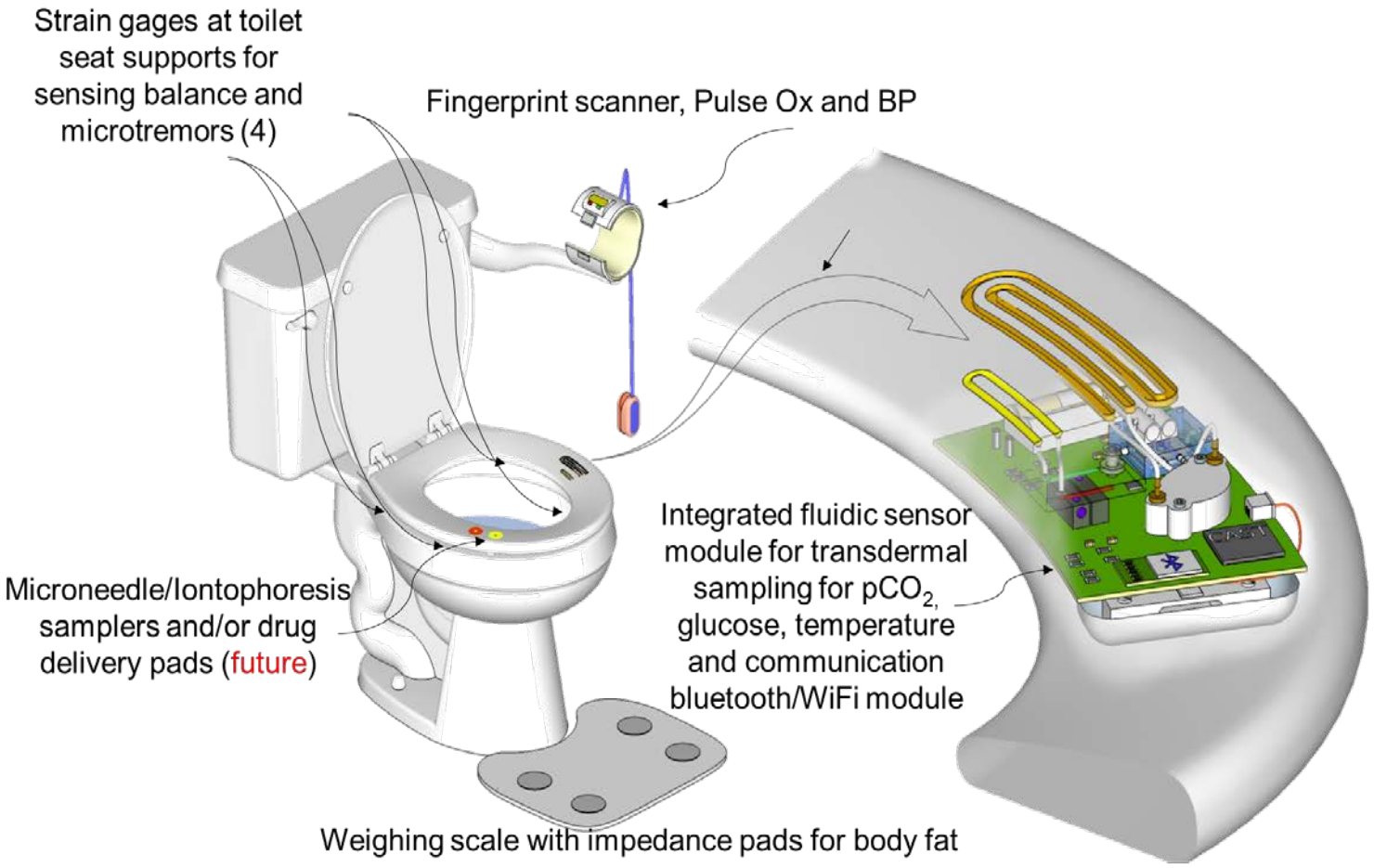
Plot of transcutaneous diffusion rates vs. arterial blood measures of O_2 and CO_2 along with a line fitted using a quadratic regression model. Normal $paCO_2$ range is 40- 50 mm Hg and hypocarbia is <35 mm Hg, hypercarbia is >55 mm Hg. The normal paO_2 range in adults is 80-100 mm Hg at sea level. For preterms, the risk for retinopathy of prematurity is when paO_2 >80 mm Hg.

Adherence (Active)/Compliance (Passive) Problems Increase Costs by $350 Billion/yr

Unintentional	**Intentional**
Forgetting	Mistrust
Shift work	Fear of side effects (actual or perceived)
Confusion	Cost
Work restrictions	Mental illness
Mental illness	Lack of belief in benefit
Memory loss	Fear of dependency
Lack of time	Fear that medication is dangerous
	Lack of desire
	No apparent benefit

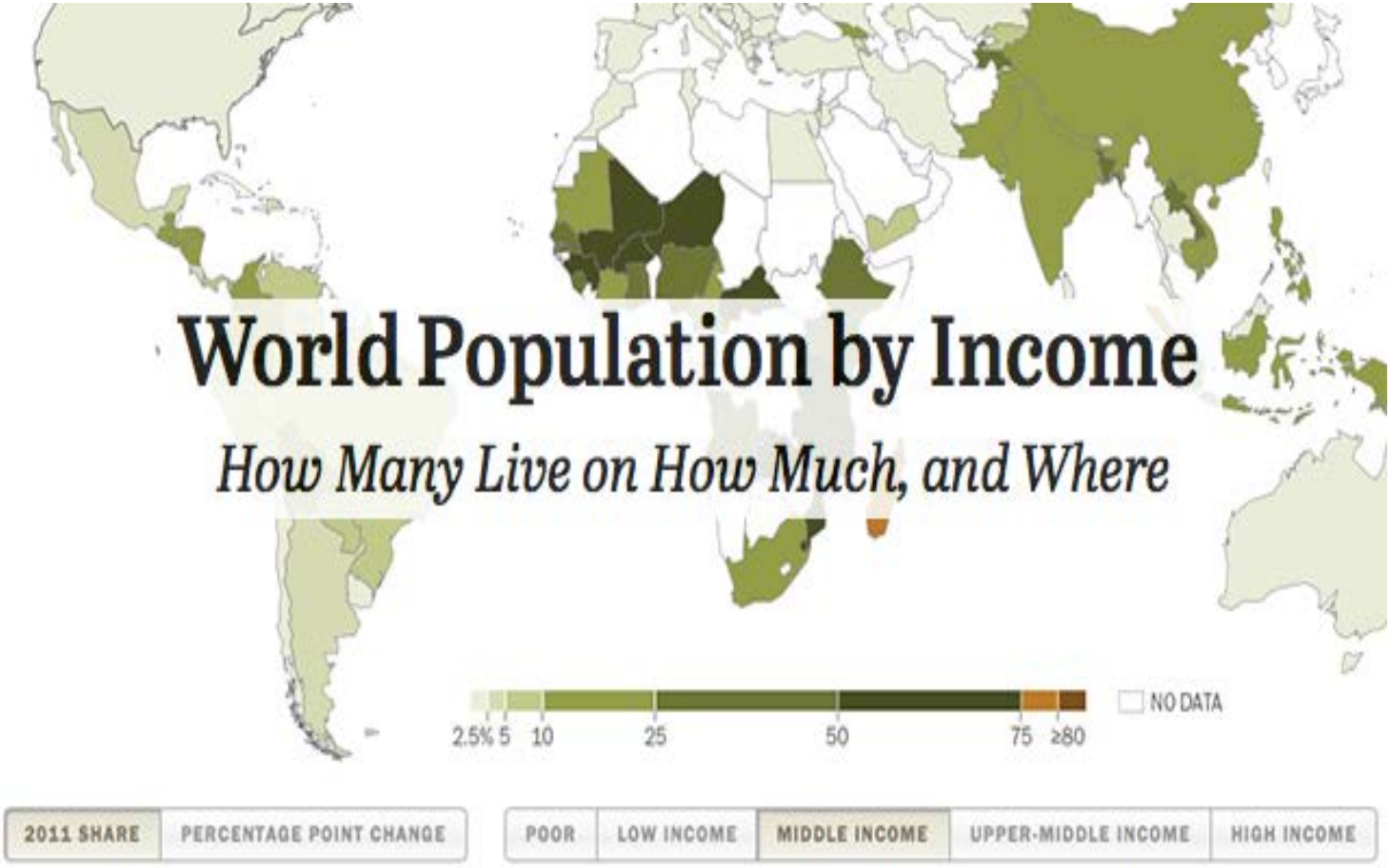
Table I. Behavioral factors responsible for patient lack of adherence to physician advice and/or compliance with treatment regimen.
https://www.aafp.org/fpm/2013/0300/p25.html#fpm20130300p25-b2

How to improve? Integrate Health Status Monitoring Into Daily Routine



Testing Underway!

Population living on <$10/day or 71% of the World Population (5 Billion)
55.7% in US are High Income, defined as >$50/day (NOT a typo, Pew Research!)
CURRENT PRICING FOR HEALTHCARE IS UNSUSTAINABLE AND IRRATIONAL

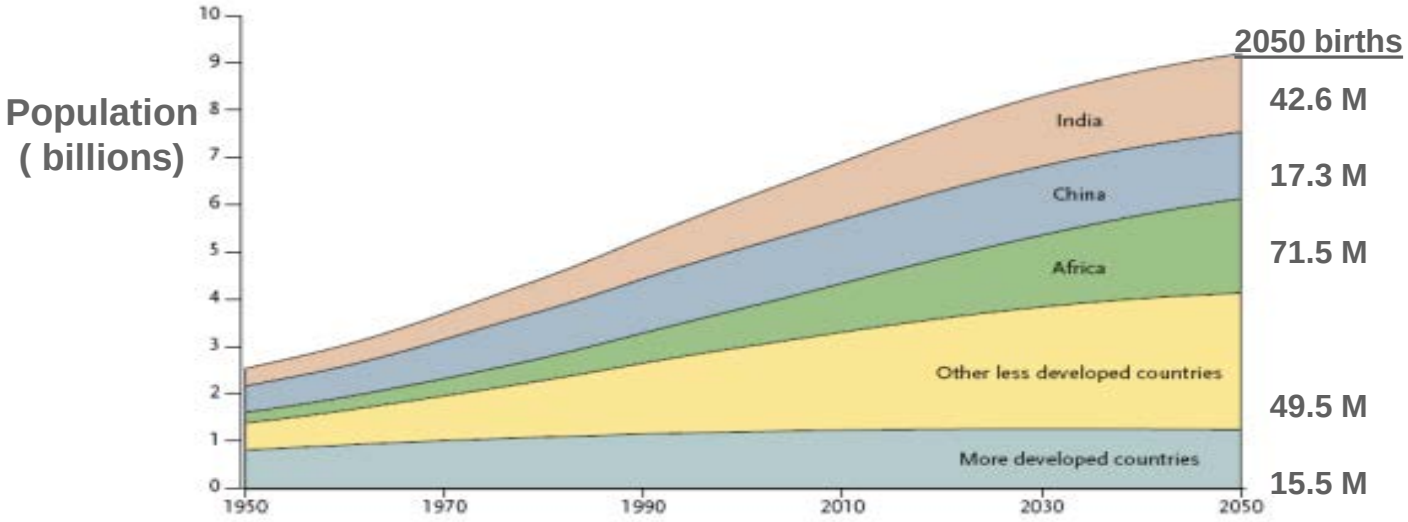


Maternal and infant care

Clinical & demographic drivers

2010 maternal & child health statistics*

- >350K mothers / yr die during pregnancy or childbirth
- 3.1M infants / yr die in neonatal period (1st 4 weeks of life), which doesn't include 3.3M stillborns
- 60-80% of newborn deaths occur in low birth weight babies
- Prematurity rate 10-12% worldwide**
- 66% of newborn deaths occur in South Asia & Africa



Top reasons for mortality

- Preterm births
- Asphyxia
- Maternal hemorrhage
- Infections for both

Aligned with MDG

Goal 4
Reduce Child Mortality

Goal 5
Improve Maternal Health

*Data: The Lancet, Volume 375, Issue 9730, Pages 1988 - 2008, 5 June 201

** WHO World Health Reprt – 2008: Mother & Child Health

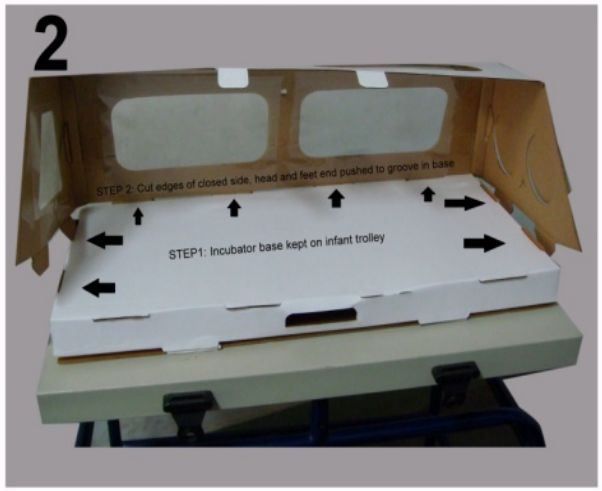
Visiting Karuna Trust Primary Health Care Center

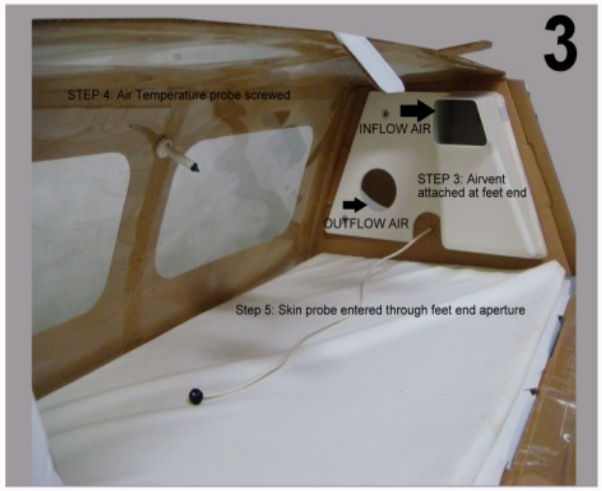
Physician expressing the need for low-cost incubator/warmer

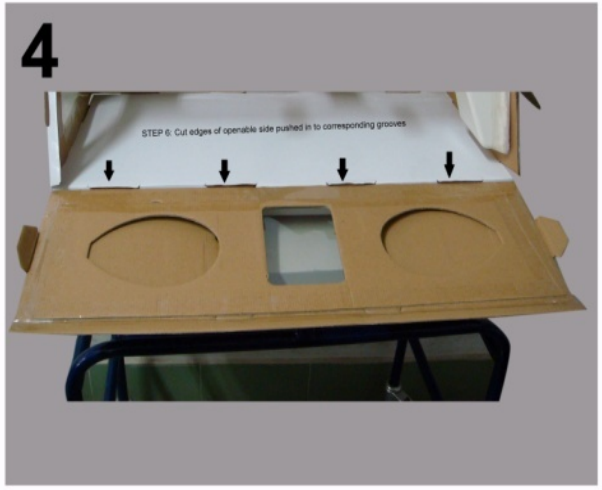
Interactive Survey With Healthcare workers- Idea for Home Use Born Here

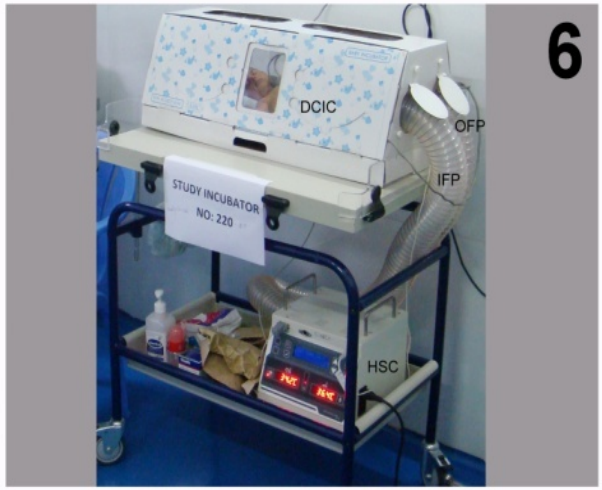
Phoenix Partnership Secured; 10% over Cost (Manufacturing/Sales/Distribution/Support)

Methods – Incubator Preparation





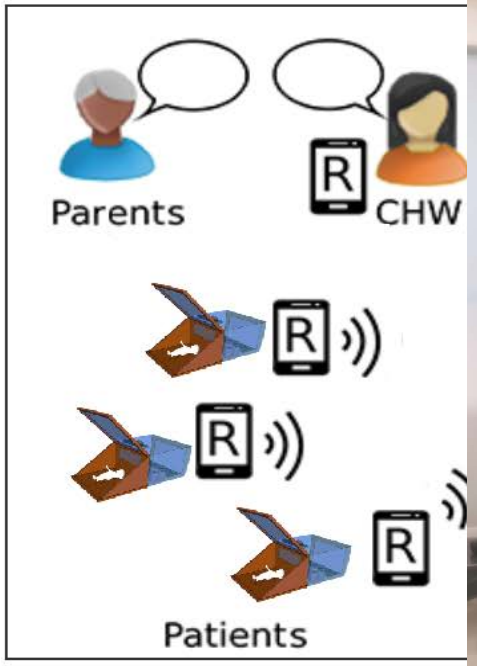





BABY INCUBATOR
PHOENIX
UMBC
Ready to use
STUDY INCUBATOR
NO: 223
Similac NeoSure

Baby weight and image transmitted

Evolution of des n- seek partnership with



Project concept. Low-cost sma e and weight to health care providers who monitor routinely etected and reported back via community health worker (CHW er mortality and malnutrition and better health outcome.


SYSTEM DESIGN MATERIALS
ITEM
Debubbler Fishers

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Q/C/C Welcome