SANDIA BIOSCIENCE

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SAND2015-3769C

Automated Preparation of Nucleic Acids from Blood for Point-of-Care Applications

Jebrail MJ, Sinha A, Renzi RF, Van De Vreugde J, Gondhalekar C, Schoeniger JS, Patel K, Branda SS

PROJECT BACKGROUND & OVERVIEW

Bioweapons & emerging infectious diseases pose formidable & growing threats to national security & public health. The key to containing & eradicating an outbreak is rapid identification of index cases & initial clusters of affected individuals. This depends upon establishment of a biosurveillance network that effectively reaches the outbreak site, even when located in remote, low-resource settings.

We are developing a deployable sample processing platform that immediately stabilizes the RNA information content of clinical & animal specimens as cDNA products that are formatted for compatibility with PCR, microarray, & Next Generation Sequencing (NGS) based diagnostics. Thus far, we have generated two fully operable platform modules enabling:

1) Extraction & purification of total RNA from finger-stick quantities of human whole blood; and
2) Microscale synthesis of cDNA compatible with PCR & NGS.

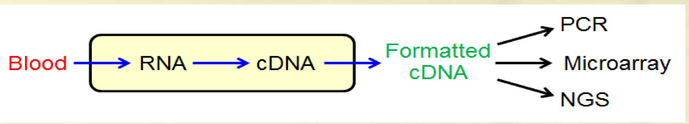
We have demonstrated that the output of the first platform module (RNA extraction/purification) can serve as the starting material for the second platform module (cDNA synthesis). Current efforts are focused on development of a fully integrated system that is fieldable.

Sample Prep + Detection: Integrate?

Pros
- Seamless Workflow
- No User Intervention
- Output → Input Match

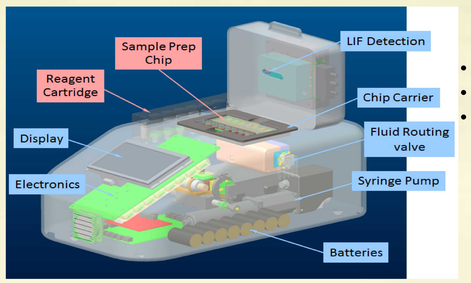
Cons
- Inflexible Workflow
- Intermediates Inaccessible
- Monolithic Redesign

Prep of Blood RNA for POC Diagnostics



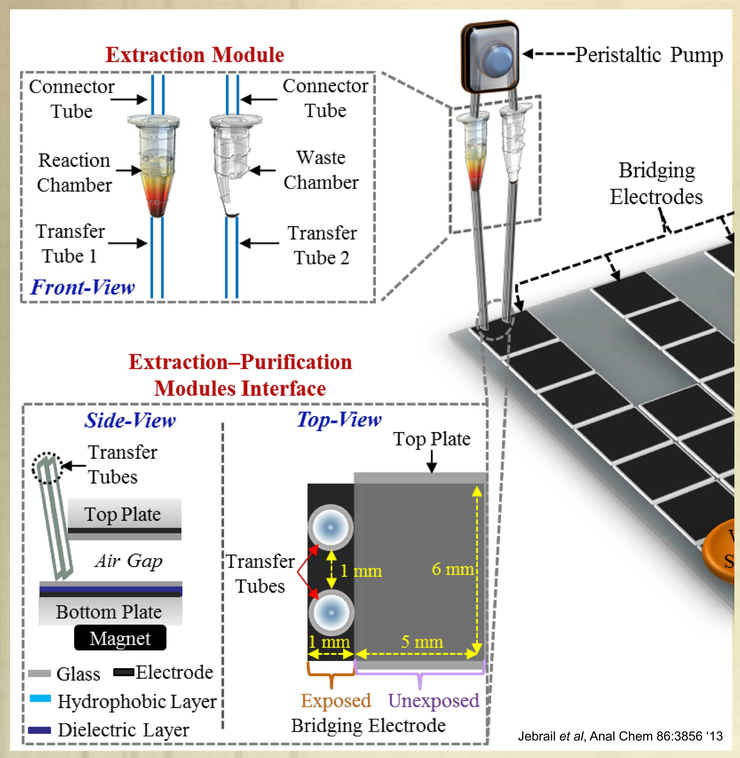
- Stabilize information content of specimen
- Format output for multiple detection methods
  - PCR for usual suspects, microarray for rare but known, NGS for unknowns
- Automate & contain sample processing
  - Protect user from sample (safety), and sample from user (quality)
- Field-forward compatible
  - Low power, no cold chain, small, rugged, cheap hardware & reagents

Design of Fully Integrated System

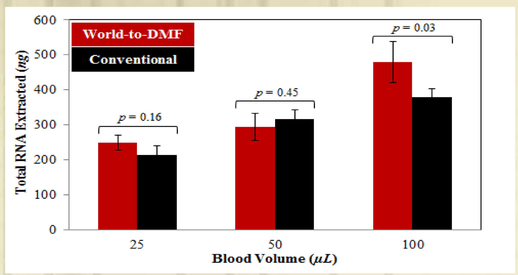


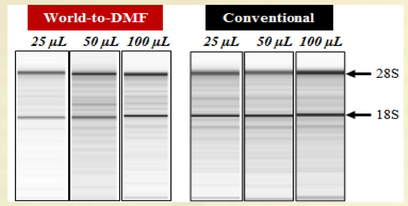
- 8” x 12” x 5”
- 8 hrs on laptop battery
- Disposable prep cassette
  - 8-plex processing
  - Injection-molded features
  - PCB DMF board
  - Plug-and-play connects
  - Cheap (~$5/prep)

BLOOD RNA EXTRACTION/PURIFICATION MODULE

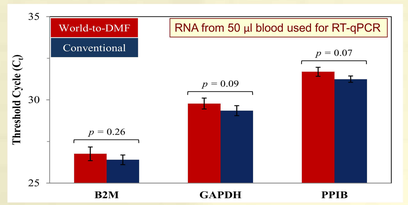


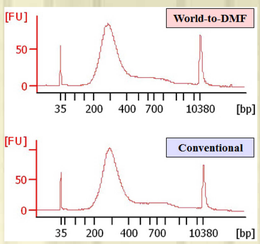
Jebrail *et al*, Anal Chem 86:3856 ‘13



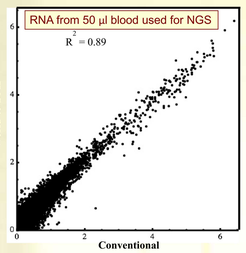


	World-to-DMF			Conventional		
Volume (μL)	25	50	100	25	50	100
A_{260}/A_{280}	1.65 ± 0.04	1.94 ± 0.12	1.99 ± 0.21	1.98 ± 0.17	1.89 ± 0.03	1.70 ± 0.14
RIN	6.8 ± 0.5	6.9 ± 0.7	6.2 ± 0.5	7.1 ± 0.4	6.5 ± 0.9	6.7 ± 0.3

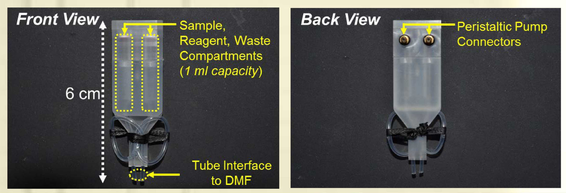




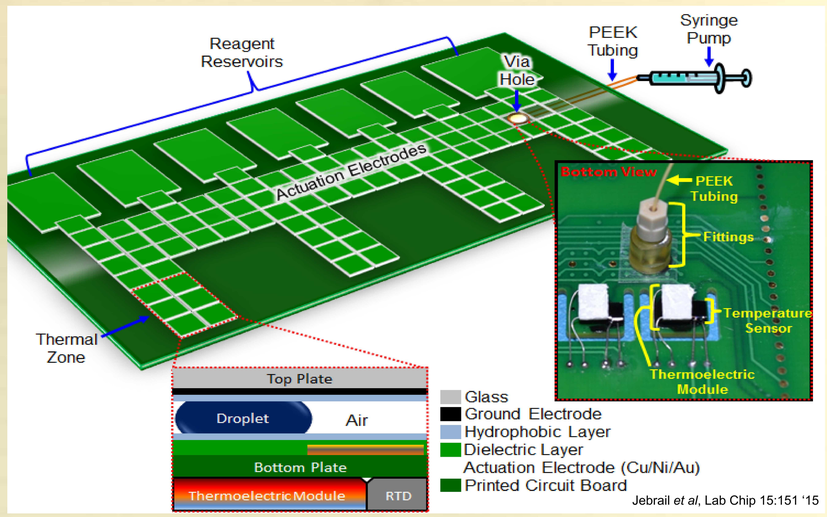
Blood Specimen	RNA Preparation	cDNA Library Yield (ng)	Raw Reads (Millions)	Passed Qfilter (% of Raw)	Mapped to Human (% of Qfilter)	R^2 Value
1	World-to-DMF	83.2	2.8	95.9	99.0	0.89
			2.8	95.9	99.0	
	Conventional	89.6	3.1	95.5	99.0	0.89
			3.0	95.5	99.0	
2	World-to-DMF	110.0	2.4	96.0	99.4	0.84
			2.3	96.0	99.4	
	Conventional	81.6	3.4	95.1	98.9	0.83
			3.4	95.2	98.9	
3	World-to-DMF	72.0	2.8	95.7	98.8	0.87
			2.8	95.7	98.8	
	Conventional	81.6	2.5	95.6	99.2	0.87
			2.4	95.6	99.2	



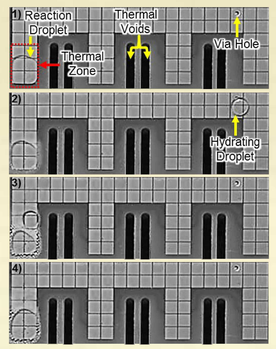
Currently testing second-generation module

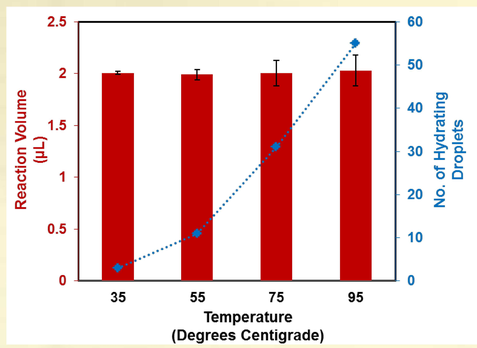


cDNA SYNTHESIS MODULE

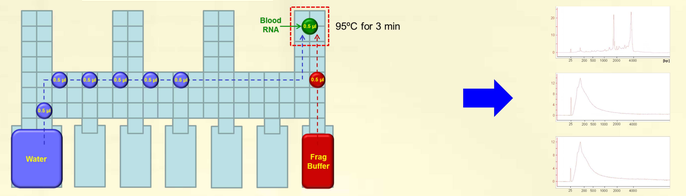


Jebrail *et al*, Lab Chip 15:151 ‘15

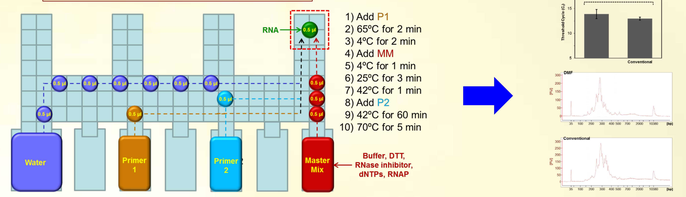




RNA Fragmentation



First Strand cDNA Synthesis



FUTURE DIRECTIONS

- Produce & test fully-integrated system
  - Pathogen-spiked human & animal blood
  - Infected human & animal blood
- Deploy locally to detect known pathogens directly and/or indirectly (*via* host response)
  - LLNL BSL-3 & animal facilities (collaborator: Sahar El-Etr)
  - UC Davis POC Testing Center for Teaching & Research (collaborator: Nam Tran)
- Deploy further afield for rapid on-site diagnosis of infection by unknown pathogens
  - ICU, mobile clinic, & field-forward settings

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