New diagnostic approaches to detect *Salmonella* spp. in blood

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Introduction

- Blood culture, the standard diagnostic, is often unavailable in the places where it is needed most

- The median *S.* Typhi count in blood is ~1 CFU/ml and ~63% are intracellular (mononuclear cells)

- Sensitivity of buffy coat culture = blood culture

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New *Salmonella* Diagnostic Assays

- Antibody-in-lymphocyte-supernatant (ALS) assay - F. Qadri
- Loop-mediated isothermal amplification (LAMP)- A. Pollard
- Microwave-accelerated metal-enhanced fluorescence

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Microwave-Accelerated Metal-Enhanced Fluorescence (MAMEF) incorporates 2 technologies

1) Metal-Enhanced Fluorescence (MEF): increases the **sensitivity** of fluorescence-based assays

2) Low-power microwave heating: kinetically accelerates the recognition events thereby **reducing** the run time

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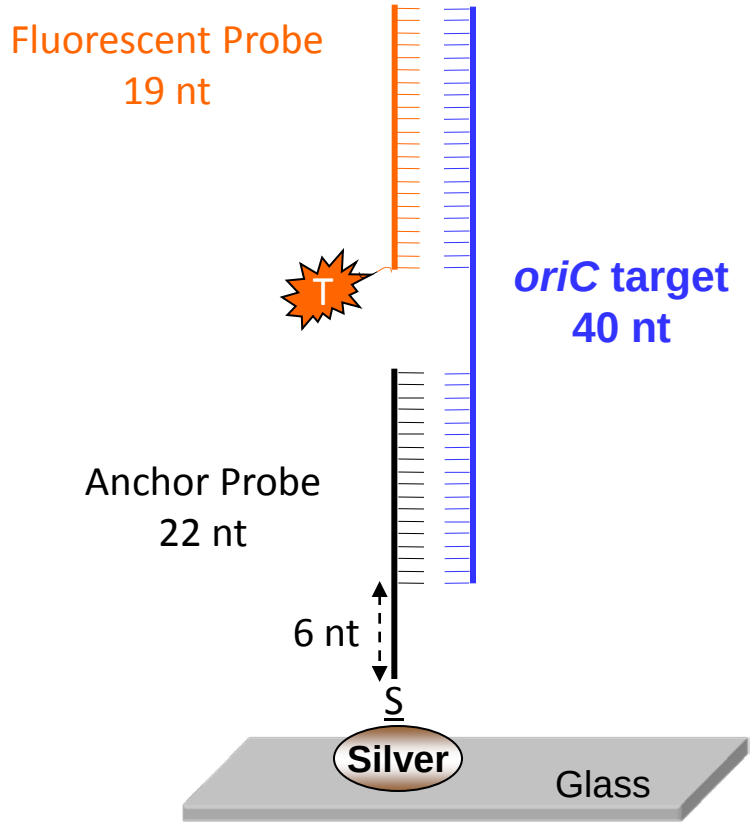
Detection of *Salmonella oriC*



SH — Anchor Probe — TAMRA — Fluorescent Probe

5′-GTTTTTCAACCTGTTTTGCGCC-3′

5′-CTTTCAGTTCCGCTTCTAT-3′

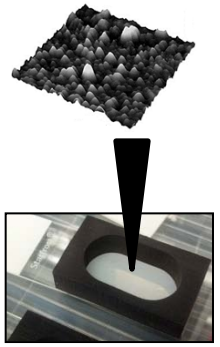
3′-GTTGGACAAAACGCGGTCGCGGAAAGTCAAGGCGAAGATA-5′

Target Probe / sequence

Microwave accelerated metal-enhanced fluorescence (MAMEF)

Lysis & release of DNA using gold bow-tie slide

SiF
Silver Island Film



Hybridization in microwave

Detection

- We have shown that we can detect 1 CFU of *Salmonella* in 1 ml of bacteriological media using MAMEF

Congealing of blood

Tennant et al. PLoS One, 2011

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General Procedure for *Salmonella* Lysis and Detection

Whole Blood

Remove RBCs/clotting factors

Lyse and release *Salmonella* DNA

Detection

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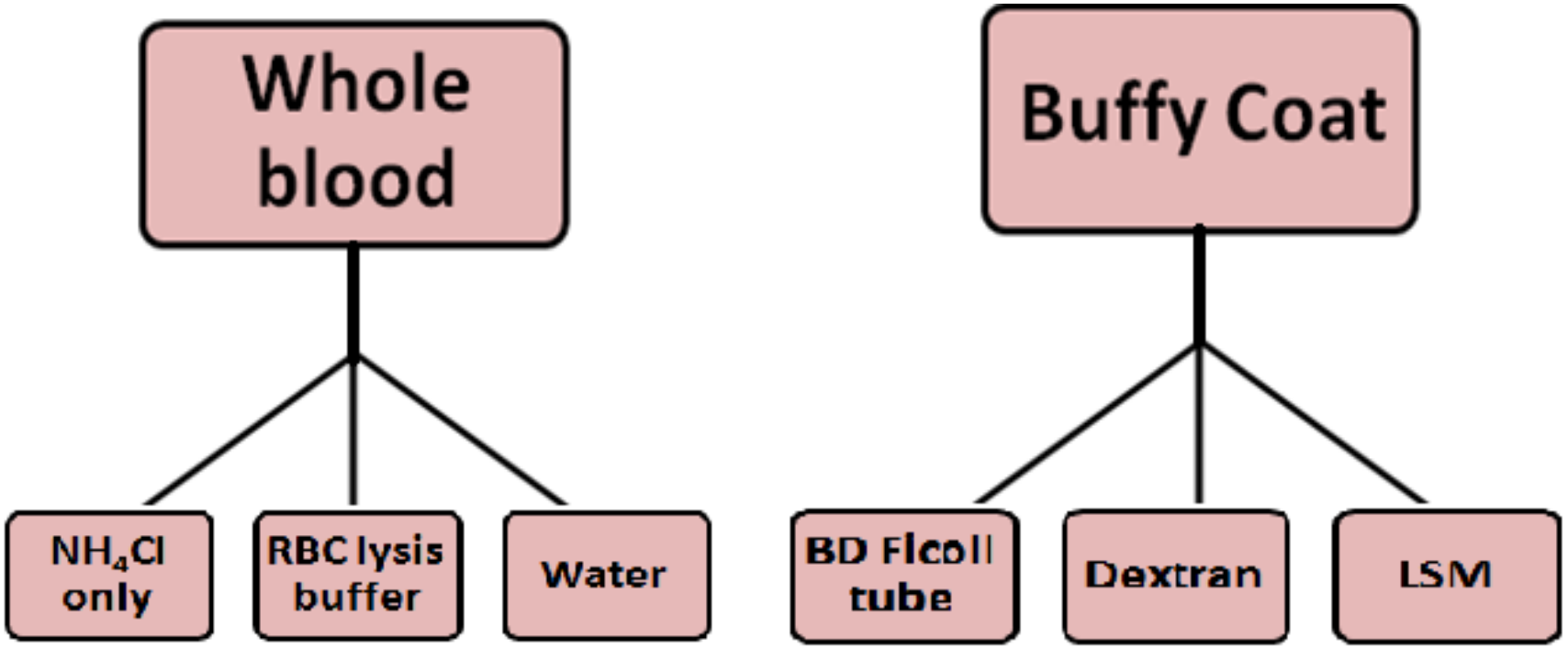
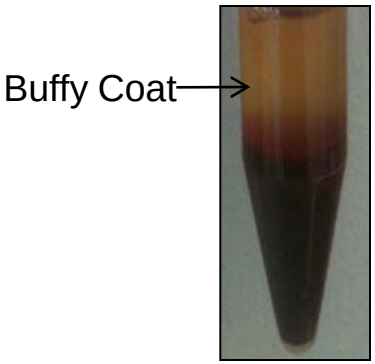
Overview of Separation Methods



8.26 g ammonium chloride (NH4Cl)
1 g potassium bicarbonate (KHCO3)
0.037 g EDTA
1 L water



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Salmonella Whole Blood Separation protocol

Anti-coagulated whole blood

↓

Add 10X volume lysis buffer

↓ Invert 10 times. Leave at RT for 2 mins

Spin at 400 *x g* for 5 mins

↓

Resuspend pellet in lysis buffer

↓ Immediately spin again 400 *x g* for 5 mins

Resuspend pellet in X ml

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Pros & Cons of best blood treatment methods

	Pros	Cons
RBC Lysis Buffer	✓Cheap ✓Fast ✓Easy ✓Intracellular & some extracellular bacteria ✓Fresh or older blood ok	✓Need swing bucket centrifuge 
Dextran	✓Cheap ✓Fast ✓Easy ✓Intracellular & some extracellular bacteria ✓Microcentrifuge	✓ Fresh blood better than old blood

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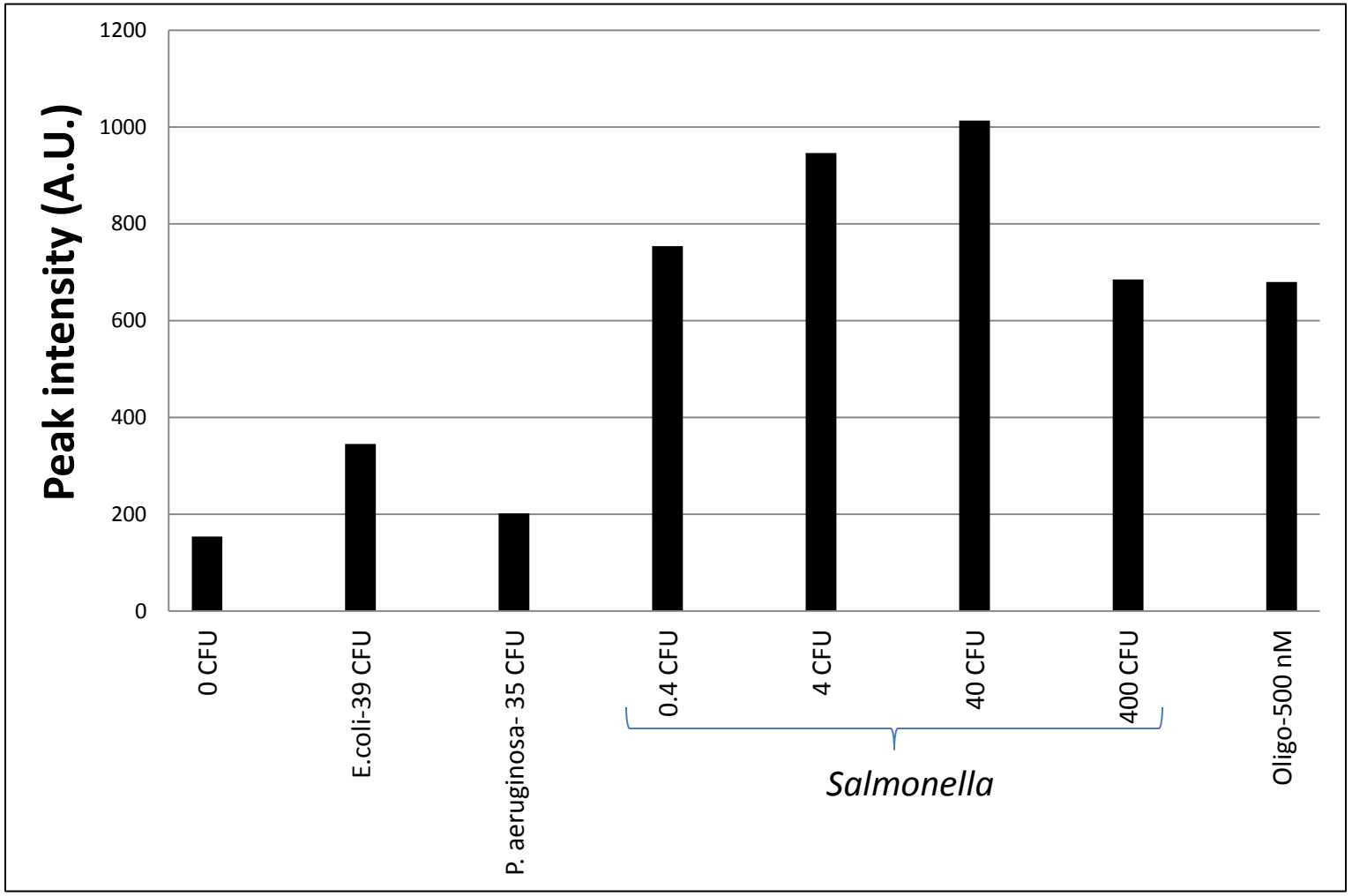
Detection of *Salmonella* in blood by MAMEF



➢ Detected 0.4 CFU *Salmonella*

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Real-time PCR detection of *S.* Typhi

- We are using a probe set described in Nga et al. (BMC Infect Dis 2010, 10:125)
- STY0201-putative fimbrial-adhesion protein
- Probes were specific and had a detection limit of ~100-200 organisms per ml of whole blood

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Using RBC lysis buffer and a mini DNA extraction kit we can detect ~0.5 CFU in 2 ml blood by qPCR 60% of the time

S. Typhi CFU per 2 ml blood	# of positive assays/total # of assays	Range of CT values
2400	1/1 (100%)	27-28
240	1/1 (100%)	29-30
56	5/5 (100%)	34-39
5.6	4/5 (80%)	36-39
0.56	3/5 (60%)	36-39
0.08	0/2 (0%)	
0	1*/5 (20%)	

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Conclusion

- We have prevented congealing of blood during microwave heating in MAMEF by removing RBCs and clotting factors
- MAMEF needs further optimization of metal surfaces before it can be field tested
- We have adapted our blood preparation method to improve sensitivity of qPCR
- Further optimizations need to be done to ensure reproducible detection of low concentrations of *Salmonella* by qPCR

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Acknowledgements

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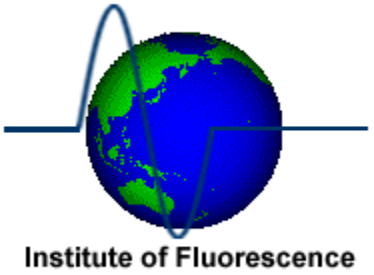
Mary A. Boyd

Deanna Toema

Sofie Livio

James Galen

Mike Levine



Institute of Florescence (UMBC)

Johan Melendez

Yongxia Zhang

Chris Geddes

Method to Improve Sensitivity

2 ml Whole Blood

RBC Lysis

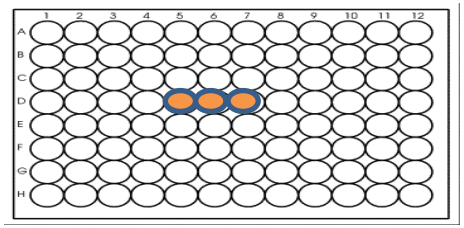
White Blood Cell (WBC) Pellet

Resuspend pellet in 200 ul buffer w/bacteria

WBC+Bacteria in 200 ul

Extract total DNA with Qiagen Blood Mini kit and Elute in **50 ul**

50 ul purified DNA



Run **9.2 ul** of sample in **20 ul** qPCR total volume (triplicate)

Each reaction is now run with ~1/5th of the sample (compared to 1/60th)

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Using RBC lysis buffer and a mini DNA extraction kit is cheaper and faster than using a midi DNA extraction kit

	Regular DNA extraction method	New DNA extraction method (CVD)
Pre-processing	None	RBC lysis
DNA extraction kit	QIAamp DNA Blood midi kit (Qiagen)	QIAamp DNA Blood mini kit (Qiagen)
Processing time	~5 h	<2 h
Cost (USD)	$8.23 per sample	$2.37 per sample (plus <$1 for RBC lysis)
Equipment required	Waterbath, centrifuge that can attain 4500 *x g*	Microcentrifuge, waterbath, benchtop centrifuge that can attain 300-400 *x g* (for RBC lysis)
Elution volume	300 ul	30-50 ul