



Role of digital PCR in high accuracy measurement in infectious disease

Dr. Denise O'Sullivan
LGC

Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance

EMPIR



The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

AntiMicroResist. Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance



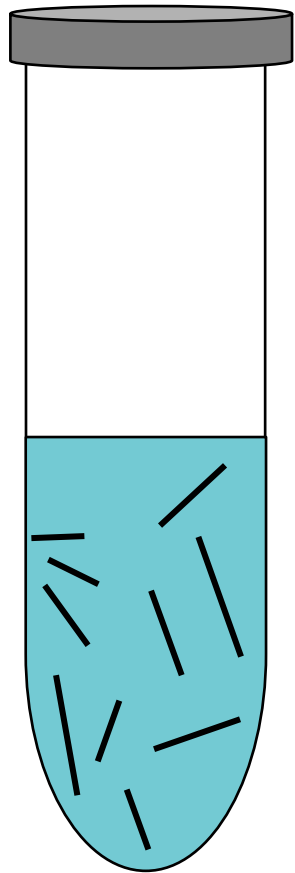
- AntiMicroResist is a clinically focussed EU consortium applying metrological concepts to develop quantitative higher order methodologies & materials to support:
 - improved application of existing molecular diagnostic testing for detection and management of AMR
 - translation of novel diagnostic methodologies required to tackle the latest challenges associated AMR



Digital PCR



qPCR $1 \times 20 \mu\text{l}$ reactions



Split sample
by dilution

dPCR $20 \times 1 \mu\text{l}$ reactions



- Limiting dilution
 - Some reaction contain 0 templates
- PCR performed as normal using standard real-time PCR chemistry
- Absolute quantification
 - +ve or -ve reactions
 - Poisson statistics to account for multiple targets per partition (> 1)

Tuberculosis



Highly Reproducible Absolute Quantification of *Mycobacterium tuberculosis* Complex by Digital PCR

Alison S. Devonshire,[†] Isobella Honeyborne,[‡] Alice Gutteridge,^{†,||} Alexandra S. Whale,[†] Gavin Nixon,[†] Philip Wilson,[§] Gerwyn Jones,[†] Timothy D. McHugh,[‡] Carole A. Foy,[†] and Jim F. Huggett^{*,†,‡}

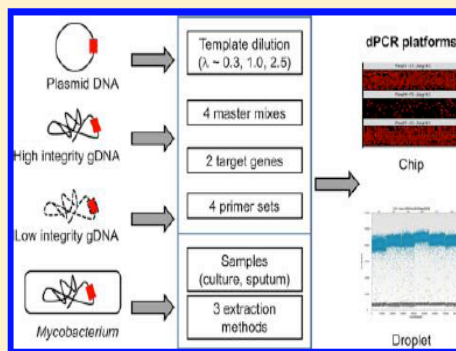
[†]Molecular and Cell Biology Team, LGC, Teddington, Middlesex TW11 0LY, United Kingdom

[‡]Centre for Clinical Microbiology, Department of Infection, Royal Free Campus, University College London, London NW3 2PF, United Kingdom

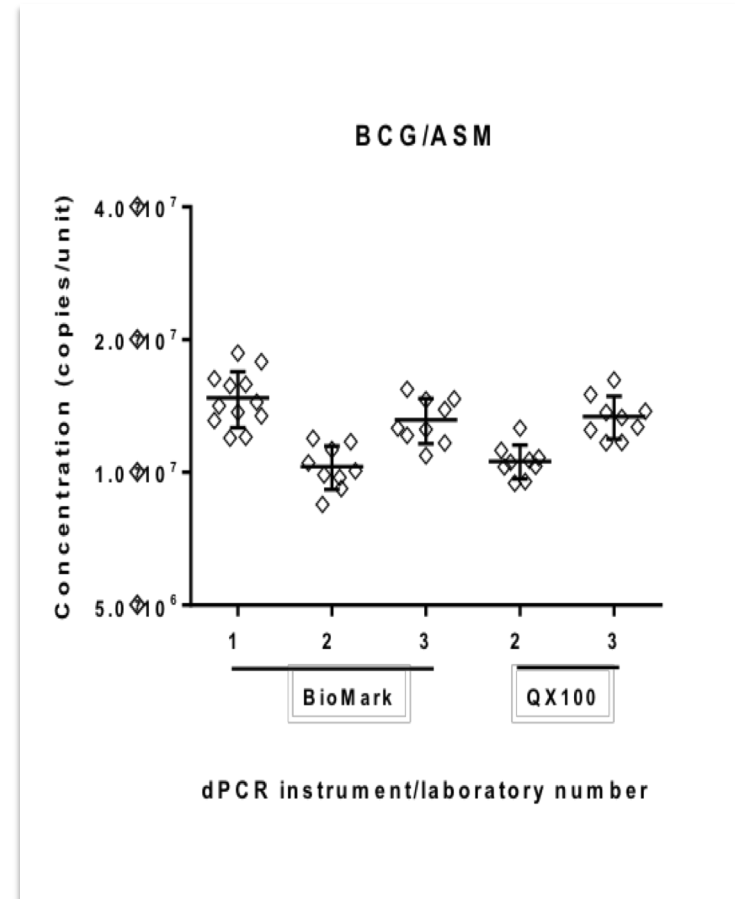
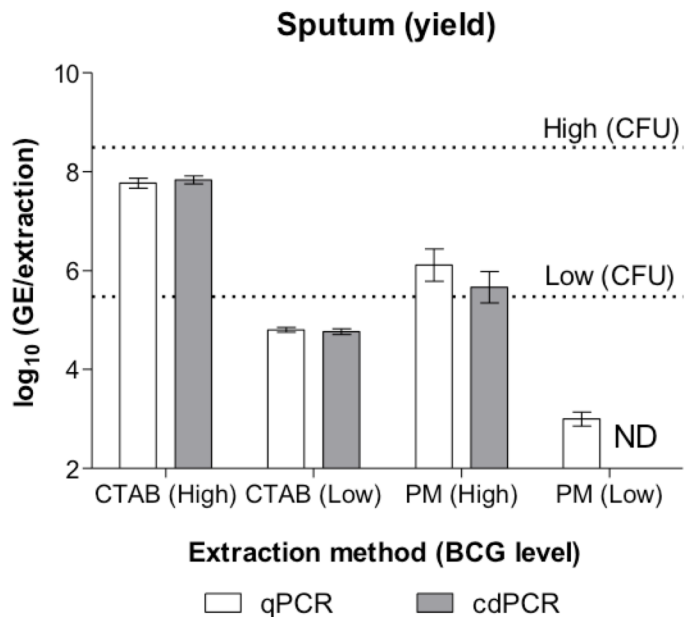
[§]Statistics Team, LGC, Teddington, Middlesex TW11 0LY, United Kingdom

Supporting Information

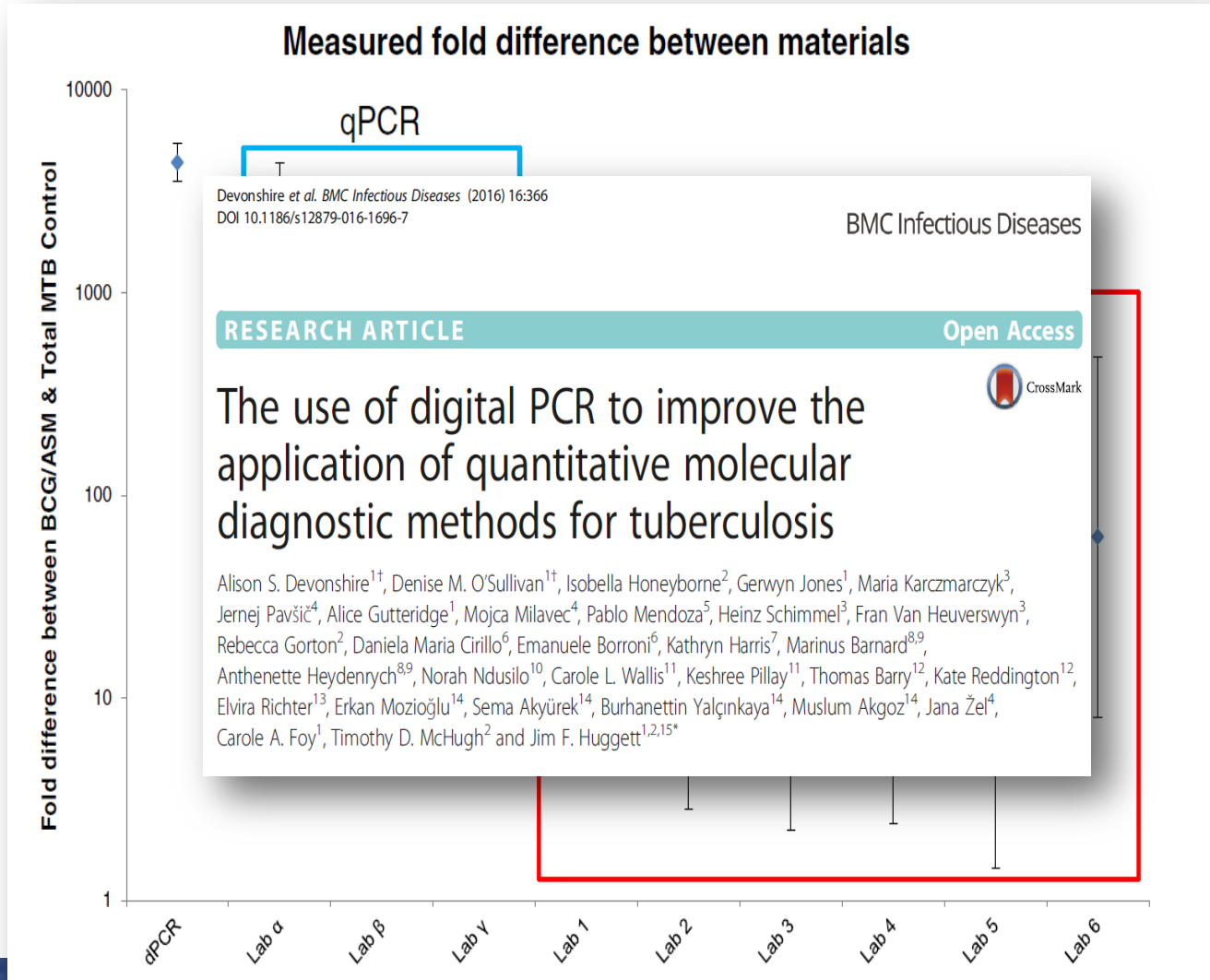
ABSTRACT: Digital PCR (dPCR) offers absolute quantification through the limiting dilution of template nucleic acid molecules and has the potential to offer high reproducibility. However, the robustness of dPCR has yet to be evaluated using complex genomes to compare different dPCR methods and platforms. We used DNA templates from the pathogen *Mycobacterium tuberculosis* to evaluate the impact of template type, master mixes, primer pairs and, crucially, extraction methods on dPCR performance. Performance was compared between the chip (BioMark) and droplet (QX100) formats. In the absence of any external calibration, dPCR measurements were generally consistent within ~2-fold between different master mixes and primers. Template DNA integrity could influence dPCR performance: high molecular



Quantification of *M. tuberculosis* using dPCR



Measured fold difference between materials



Current Work



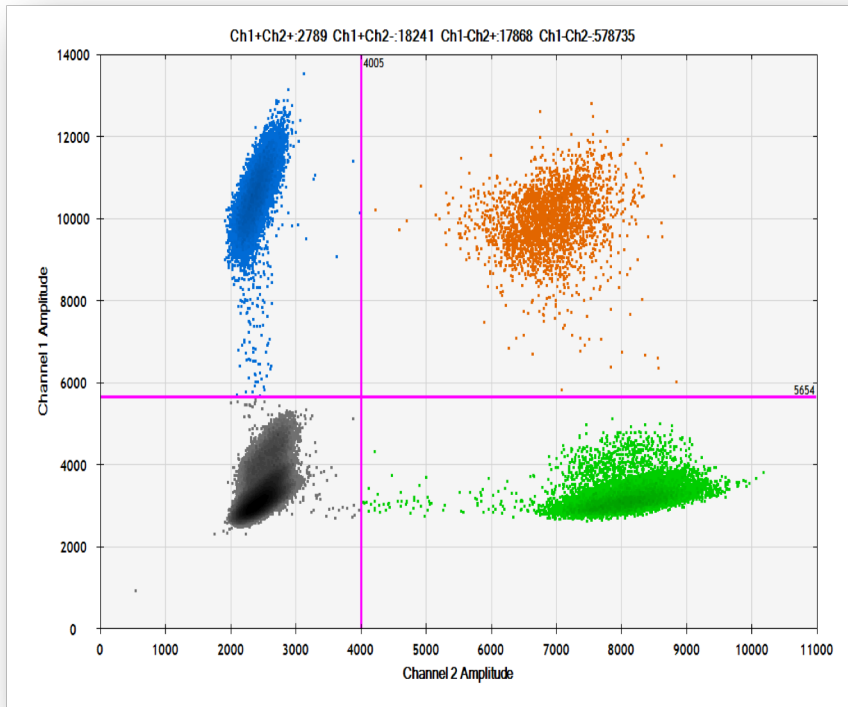
- Inter-laboratory study using more complex materials to look at molecular methods for detecting TB and associated resistance
- Study to commence Autumn 2019



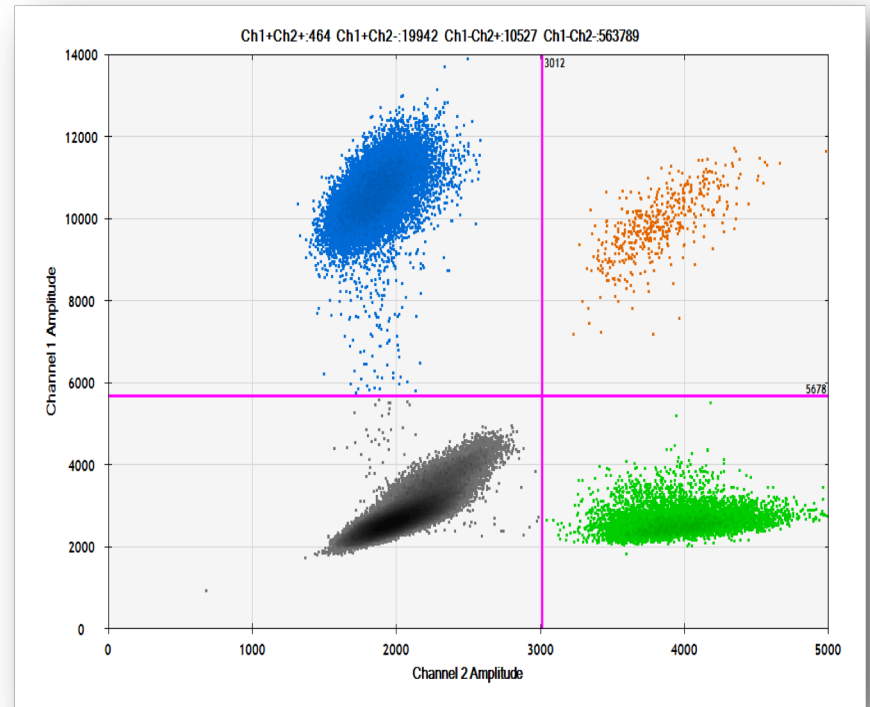
MRSA



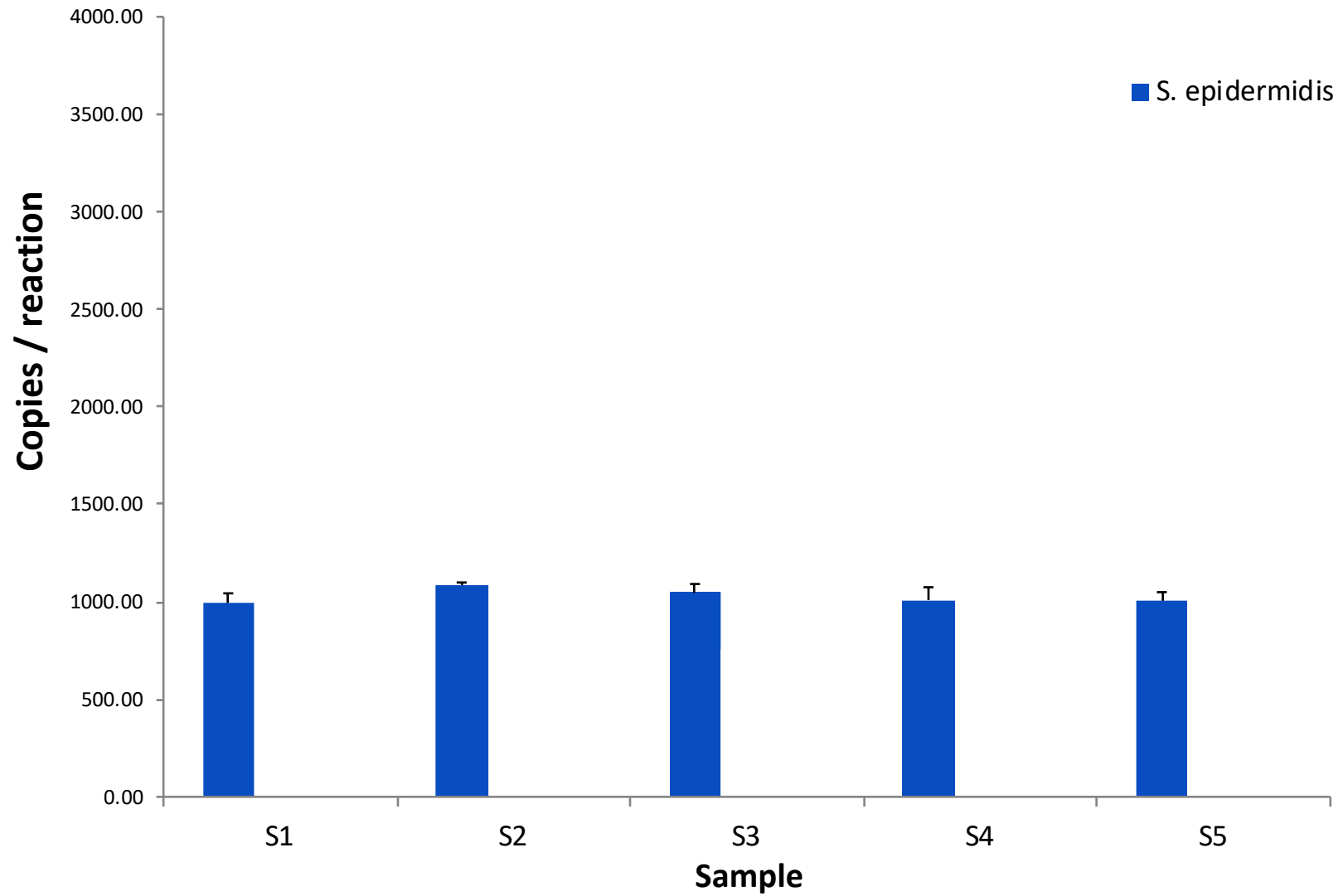
S. aureus & *mecA* assays



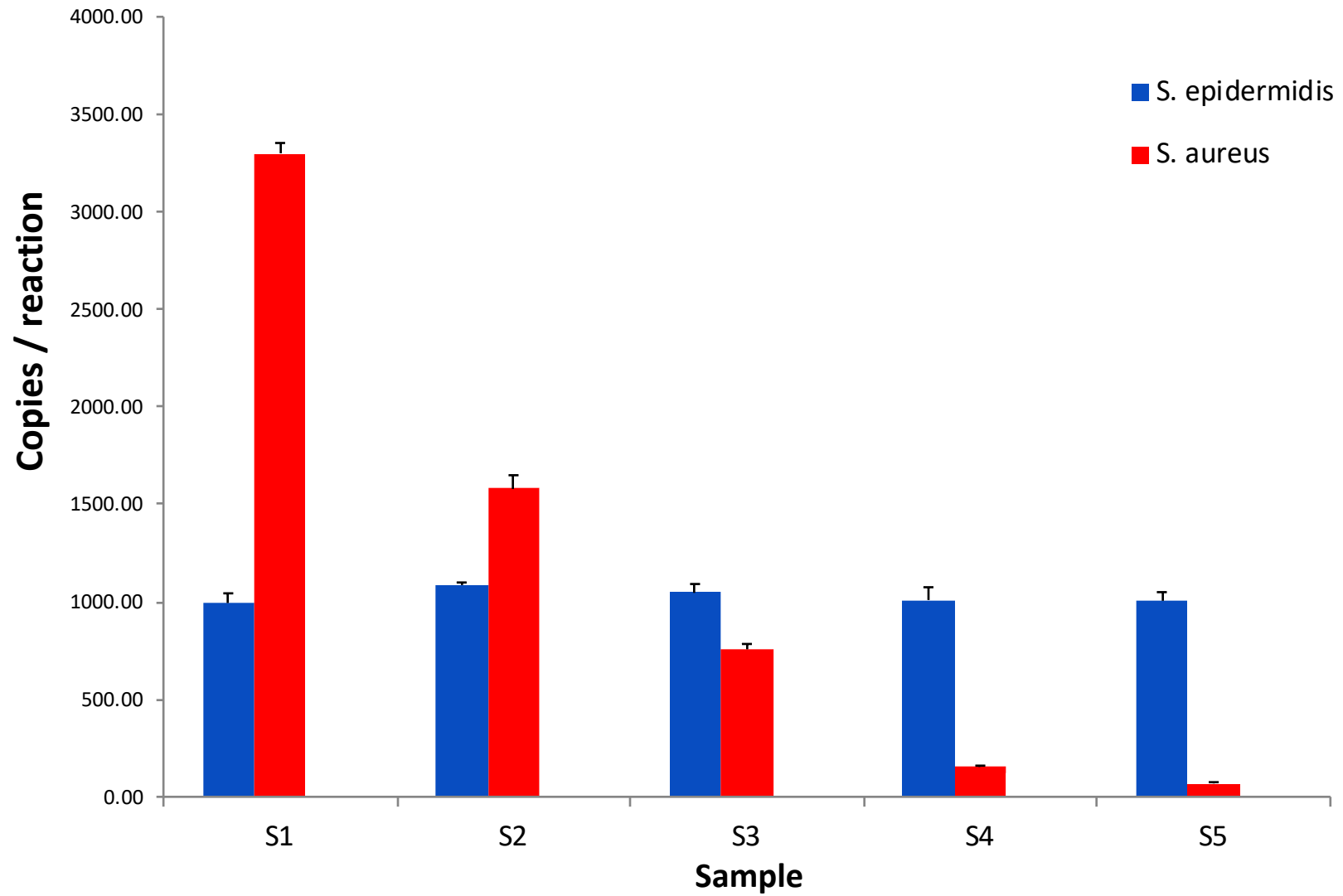
S. epidermidis & *mecA* assays



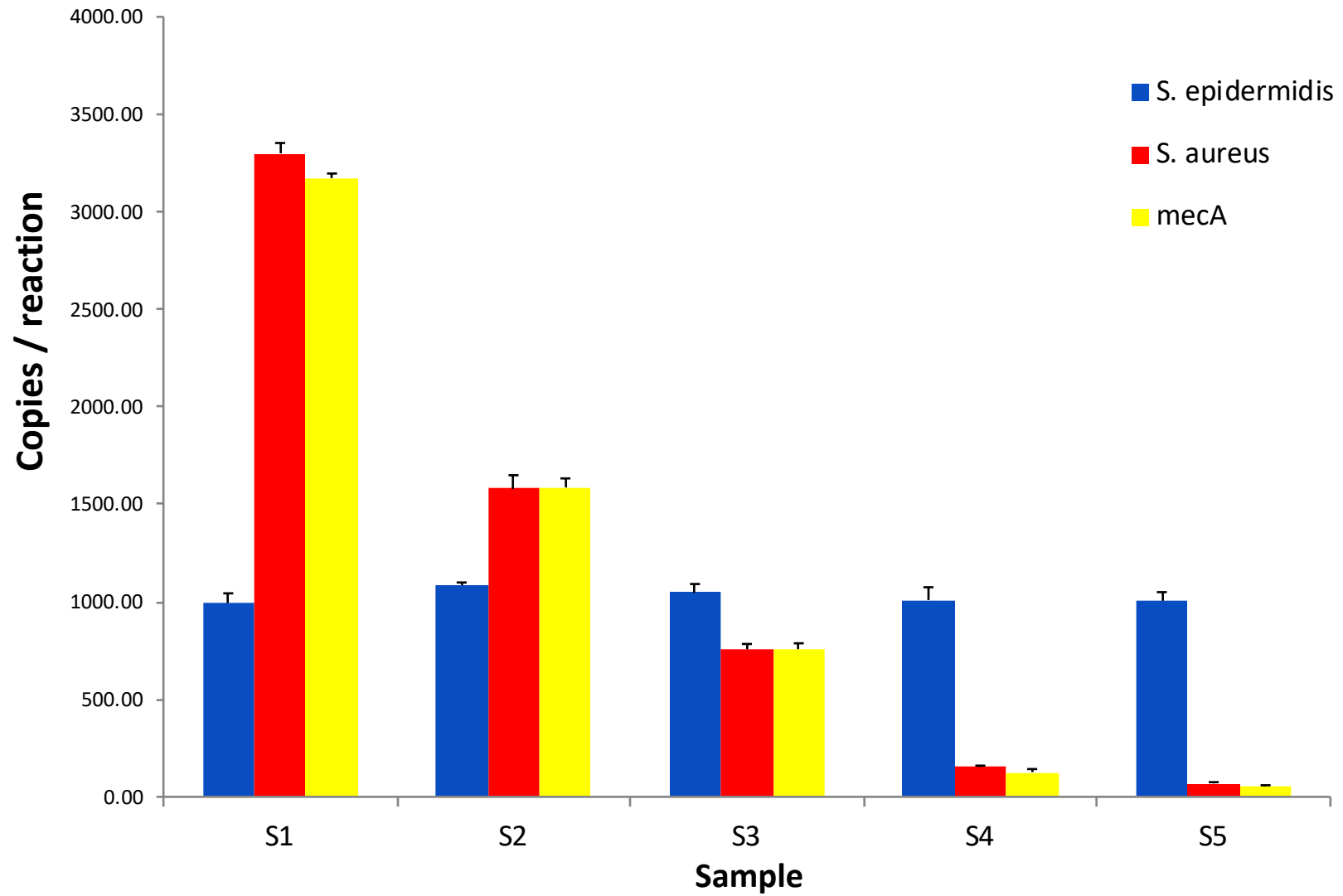
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MRSA



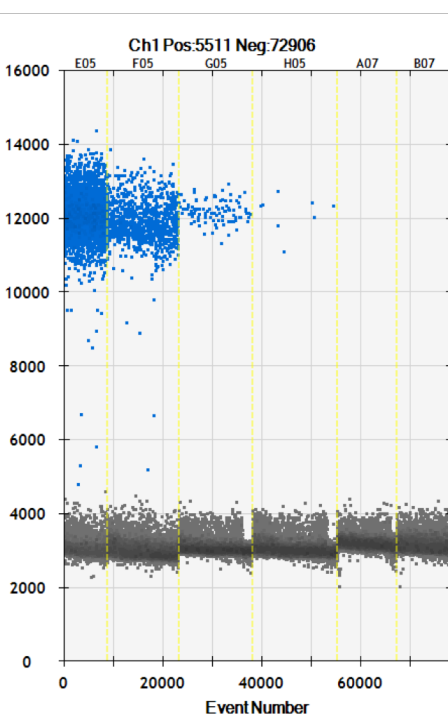
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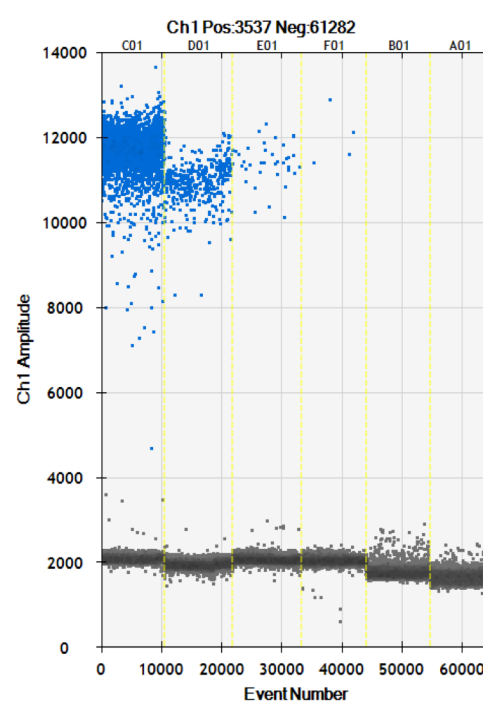
CRE Model



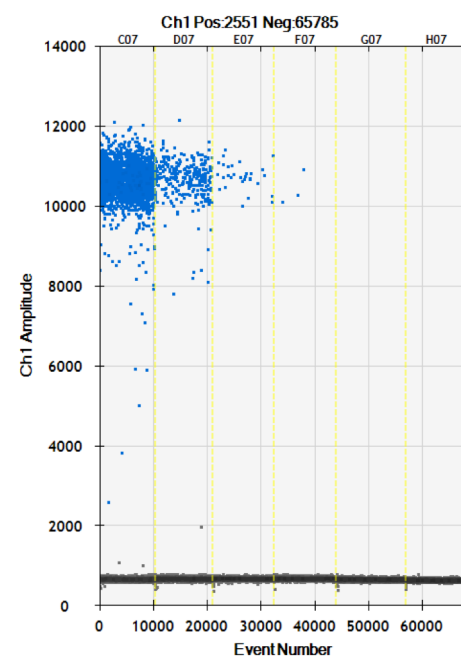
oxa48



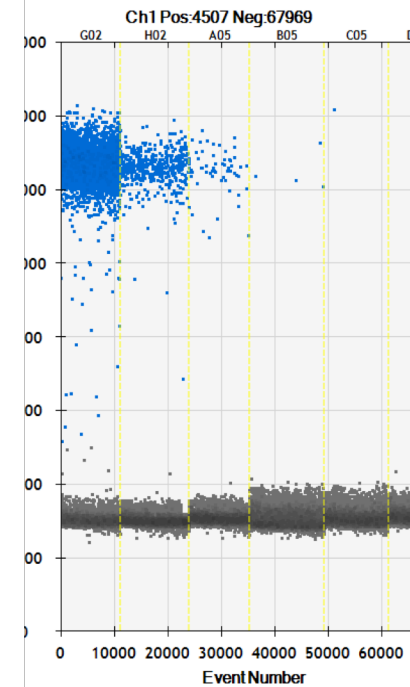
VIM



KPC



NDM



Conclusions



- dPCR offers an absolute counting method to quantify bacterial DNA without calibration
- Could assist in the preparation of bacterial reference materials needed to support growing point of care tests
- Ultimately the method may also offer an alternative molecular diagnostic tool

Next project

SEPTIMET

Metrology to enable rapid and accurate clinical measurements in acute management of sepsis



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Thank you

15HLT07 AntiMicroResist



Publishable Summary for 15HLT07 AntiMicroResist Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance

Overview

In 2014 a World Health Organisation (WHO) report stated that antimicrobial resistance (AMR) is so serious, that it threatens the achievements of modern medicine, and while new therapies to treat resistant pathogens are needed, the diagnostic tools required to guide their application are equally lacking. This clinically focussed project will apply innovative metrological concepts for developing quantitative higher order methodologies and materials to support the improved application of diagnostic testing to the detection and management of AMR.

15HLT07 AntiMicroResist



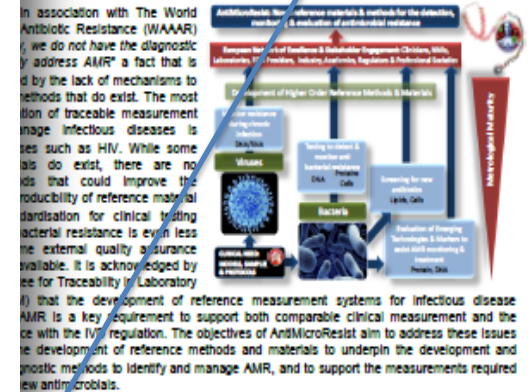
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Antimicrobial resistance (AMR) is a global public health threat. The WHO estimates that AMR will account for ~25,000 European deaths per annum. A recent review estimates AMR staggering 45 % of global deaths by 2050 (<http://am-review.org/home>). In recognition of this problem, several European activities to monitor detection and treatment of AMR have been initiated. The European Centre for Disease Control (ECDC) Interactive database for clinical antimicrobial resistance (EAR3-Net).

However, there is still a vital stakeholder need for methods to be developed and improved in order to accurately diagnose patients with infections that do need antimicrobials. Current diagnostic methods are often unreliable, and practitioners with respect to correct and effective therapies, to reduce over prescription of antimicrobials. The development of innovative antimicrobials.



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EURAMET

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denise.osullivan@lgcgroup.com