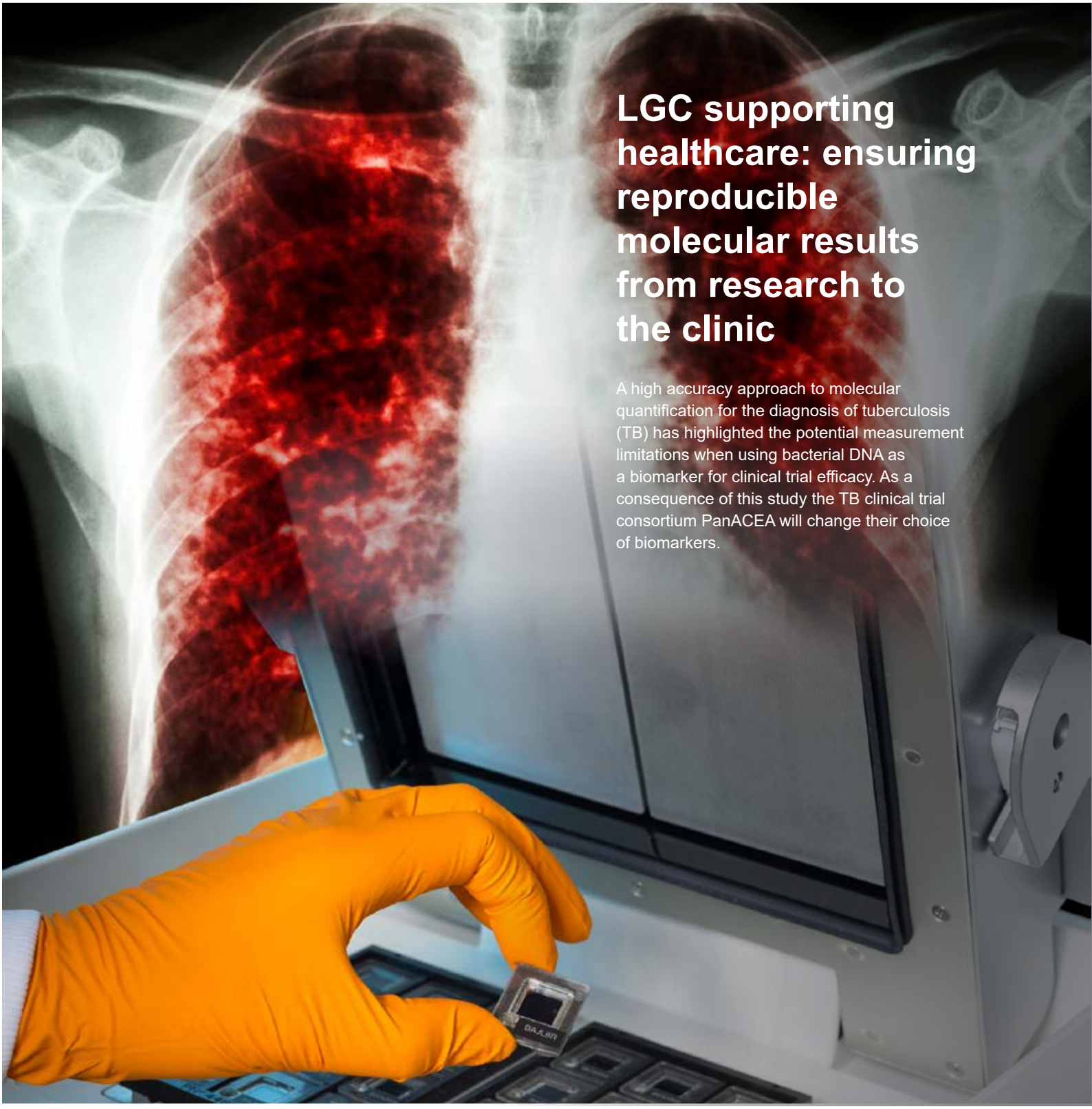

LGC supporting healthcare: ensuring reproducible molecular results from research to the clinic

A high accuracy approach to molecular quantification for the diagnosis of tuberculosis (TB) has highlighted the potential measurement limitations when using bacterial DNA as a biomarker for clinical trial efficacy. As a consequence of this study the TB clinical trial consortium PanACEA will change their choice of biomarkers.

Department for Business, Energy & Industrial Strategy

FUNDED BY BEIS

Background

Tuberculosis (TB) is one of the leading causes of preventable death worldwide, with approximately 8 million new cases each year. Mortality rates associated with TB are some of the highest associated with an infectious disease (at over 1 million deaths per year). Many patients do not complete the six month antibiotic treatments required, potentially leading to future disease reactivation and possible drug resistance.

There is a vital need for new, shorter drug regimens as well as novel biomarkers, such as bacterial DNA, to rapidly determine treatment efficacy and predict potential relapse. Quantitative molecular methods offer the potential to accurately identify the amount of bacteria present to inform clinical decisions. However, the validity and robustness of each method must be assessed prior to its effective use in the field.

An automated diagnostic test for TB (Cepheid Xpert® MTB/RIF) has been proposed to be used in a quantitative capacity to assess drug efficacy during clinical trials. This potentially enables a molecular approach to be used in remote parts of affected areas of Africa and Asia where many clinical trials take place. However neither Cepheid nor the WHO has recommended the test for quantification of the TB bacterium, only for its identification. A comprehensive evaluation of the method performance is required to determine its suitability for quantitative analysis and assess how it might be standardised between laboratories.

Impact

The highly sensitive technique of digital polymerase chain reaction (dPCR), a method based on single molecule detection and counting, offers a route for high accuracy quantification to determine the efficacy of other quantitative molecular measurements.

A dPCR reference method for accurate quantification of *Mycobacterium tuberculosis* (the bacterium which causes TB) was developed at LGC in close collaboration with the UCL Centre for Clinical Microbiology at the Royal Free Hospital. The approach was then used to accurately assign a value to two reference materials which differed by a factor of 1000 in their bacterial DNA content. These were sent to six different laboratories across Europe and Africa to quantify using the Xpert MTB/RIF test. Although all the laboratories could distinguish between the high and low materials, none of the laboratories were able to quantify the magnitude of the difference, with one group measuring a difference of a factor of 5. This demonstrates the need for further improvements before this approach can be used to determine the clinical feasibility of molecular quantification of *M. tuberculosis* during the management of TB.

These findings were presented at the European & Developing Countries Clinical Trials Partnership Forum in Zambia in November 2016 and highlighted the need for a metrological approach to support effective biomarker identification. As a result, at the recent meeting in Cape Town of the multinational TB clinical trials consortium (PanACEA), the evidence that Xpert MTB/RIF results should not be used as a quantitative measure of TB bacterial load was reviewed. It was agreed that Xpert MTB/RIF quantitative data would not be used as a biomarker in the selection of drug regimens to progress in treatment trials.

This represents a significant outcome for this study and demonstrates the potential role for reference materials in evaluating the reproducibility of molecular methods during the translational stage of method development.

Dr Klaus Reither MD/MScIH, Head of Clinical Research Unit, Group Leader Clinical TB Research, Swiss Tropical and Public Health Institute, Switzerland

"We were considering to categorise the quantity of Mycobacterium tuberculosis as a clinical biomarker by using the cycle threshold values provided by the Xpert MTB/RIF. After looking at the large variation between the cycle threshold levels from the different laboratories who received the same sample in the paper by Devonshire et al (2016), we decided that we won't use this approach to characterise the clinical samples."

Devonshire AS et al. The use of digital PCR to improve the application of quantitative molecular diagnostic methods for tuberculosis. BMC Infectious Diseases (2016) 16:366. DOI:10.1186/s12879-016-1696-7

Devonshire AS, Honeyborne I, Gutteridge A, Whale AS, Nixon G, Wilson P, Jones G, McHugh TD, Foy CA, Huggett JF. Highly reproducible absolute quantification of Mycobacterium tuberculosis complex by digital PCR. Anal Chem (2015) 87(7):3706-13 DOI:10.1021/ac5041617

For further information, contact:
LGC, Queens Road, Teddington, Middlesex TW11 0LY, UK

Tel: **+44 (0)20 8943 7393** Email: **nmshelp@lgcgroup.com** Web: **www.lgcgroup.com/nmi**

Department for Business, Energy & Industrial Strategy

FUNDED BY BEIS