concentration. However, four replicate samples using the same protocol also demonstrated a 1.7 cycle mean difference, similar to the up to 1.9 cycle difference seen across protocols. The similarity in cycle differences suggests that differences in cycle threshold values may be due to intra-assay variation and not related to the sample processing protocol used. These results show that centrifugation may not be necessary for testing CSF. Additionally, as CSF samples tend to be paucibacillary compared to sputum, less sample reagent may be needed to produce an adequate tuberculocidal effect. Further research, ideally on paired samples from TB meningitis patients, will help elucidate the most sensitive and safest method to test CSF with the Xpert MTB/RIF test, particularly in settings that may not have access to centrifugation.

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Cerebrospinal fluid testing with Xpert MTB/RIF may not require complex sample processing protocols http://ow.ly/xkGjg

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From the authors:

We appreciate the response from R.F. Luo and co-workers on our meta-analysis on the accuracy of Xpert MTB/RIF for extrapulmonary tuberculosis [1], as well as the data they present on various approaches to cerebrospinal fluid (CSF) processing for Xpert MTB/RIF testing.

In a recent guidance document on Xpert, the World Health Organization (WHO) recommend that Xpert should be used as a first-line test over conventional microscopy and culture in patients with suspected tuberculous meningitis [2, 3]. This recommendation was based on a systematic review of the evidence and expert consensus [4]. However, our systematic review noted the highly variable sample processing methods used across and within studies, and was unable to identify the best approach for sample processing. The latter is largely due to the lack of recommendations from both the manufacturer and WHO on how to process nonrespiratory samples.

WHO has recognised the need for such guidance and has published an Xpert MTB/RIF implementation manual with recommendations on the technical and operational "how to", which includes standard operating procedures for processing of CSF, lymph node samples and other tissues [5]. While this is a good step forward, the recommendations are based on expert opinion and limited experimental data on the optimisation of sample preparation comparing different protocols on the same clinical samples or spiked samples in a controlled laboratory setting.

The data by R.F. Luo and co-workers addresses this knowledge gap. In a controlled laboratory environment comparing different protocols on CSF, they were not able to reproduce the finding in our systematic review of an increased sensitivity of Xpert on CSF with a centrifugation step prior to inoculation with the sample

reagent. Their findings further suggest that for the paucibacillary type of samples, such as CSF, less sample reagent might be necessary.

These findings need to be confirmed independently and should be combined with testing of the tuberculocidal effect at lower sample-to-sample-reagent ratios before they can be recommended for clinical use. We hope that other groups will follow the example of R.F. Luo and co-workers and evaluate different protocols for different sample types, so that Xpert testing on nonrespiratory samples can be optimised.

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Improved, evidence-based standard operating procedures for processing CSF samples needed to optimise TB detection http://ow.ly/ytgfc

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Importance of concomitant local and systemic eosinophilia in uncontrolled asthma

To the Editor:

We read with interest the comprehensive, combined retrospective and prospective study on eosinophilia in asthma by SCHLEICH *et al.* [1]. As compellingly demonstrated by SCHLEICH *et al.* [1] concomitant elevation of sputum and blood eosinophil numbers is an important factor in poorly controlled asthma. These data agree with the view that both local airway "inflammation" and blood eosinophilia contribute as risk factors in asthma [2]. However, there are additional eosinophil features, other than just counts, that characterise asthma. Here we draw attention to the potential roles of primary lysis/necrosis of eosinophils as blood biomarkers and bronchial-pathogenic mechanisms, especially in uncontrolled asthma.

SCHLEICH *et al.* [1] briefly discuss previous findings on eosinophil numbers reported by VOLBEDA *et al.* [3]. However, a principal message of the latter study concerned association between poor asthma control and "activated eosinophils" and loss of "epithelial intactness", respectively, in bronchial biopsies. It emerged that the eosinophils had been activated by primary lysis that results in the spilling of toxic protein-releasing free eosinophil granules (FEGs) in the bronchial tissue [3, 4].