# Development of a 29-mRNA Loop Mediated Isothermal Amplification Assay for the Rapid Diagnosis of Acute Infection and Sepsis

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## Introduction

The rapid diagnosis of suspected acute infections and sepsis is critically needed. Current diagnostics are limited by speed, sensitivity, and/or interpretability. Early empirical antimicrobials contribute to perturbed microbiomes, adverse events, and antimicrobial resistance. The novel InSep™ acute infection and sepsis test measures 29 human mRNAs and employs advanced machine learning to 1) distinguish between bacterial and viral infections and noninfectious etiologies, and 2) predict the severity of the condition.

Currently, InSep clinical trials have quantified RNA's on the NanoString nCounter™ platform in the absence of a point-of-care instrument (in development). While highly accurate, nCounter does not provide the rapid turnaround time and low hands-on time needed for implementation in Emergency Department (ED) settings. Thus, we aimed to translate InSep into a rapid, Loop Mediated Isothermal Amplification (LAMP) based assay.

Here, we report on the development and analytical validation of InSep as a LAMP assay in comparison to absolute digital counting on the nCounter.

## Methods

- **Sample Collection:** We collected whole blood samples drawn in PAXgene® RNA blood tubes in four prospective clinical trials across the USA, India, and Europe. Samples from patients presenting with acute bacterial (n=10), acute viral (n=10) and severe (n=10) infections were used; 20 control samples were drawn from healthy volunteers.
- **RNA Extraction:** Total RNA was extracted from 1.0 mL of PAXgene blood using a rapid magnetic bead-based protocol for the LAMP assay and a modified RNeasy® Micro Kit on the QIAGEN QIAcube® platform for the "gold standard" NanoString measurements. The bead-based method utilizes home brew buffers and has been adapted for ideal purification on a point-of-care cartridge (in development). Additionally, the complete protocol takes under 15 minutes.
- **Assay Design:** LAMP assays were designed using PrimerExplorer software (v7) targeting specific isoforms found in whole blood and exon:exon junctions. Primer sets were screened for RNA specificity and off-target amplification. Linearity assessments using in-vitro transcribed artificial DNA sequences (IDTDNA) were completed. The 29 InSep™ and 3 housekeeping mRNAs were quantified with LAMP on ABI QuantStudio® thermocyclers and nCounter. Assays were discarded if slope was outside the range of 2.5 and 3.5 and if the Limit of Quantitation was found to be $>10^4$.
- **Data Analysis:** Calibrated LAMP gene expression values (derived from LAMP signal curve Tt values) and nCounter counts were analyzed using two proprietary neural network classifiers: IMX-BVN-2 which outputs a bacterial score and a viral score, and IMX-SEV-2 which outputs a severity score. Analytical validity of the LAMP assays was analyzed against nCounter results as the gold standard.

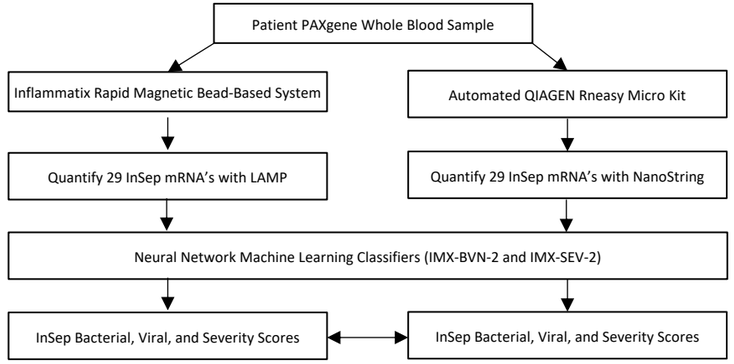


**Figure 1. Study Flowchart** for assessing analytical validity of LAMP assay compared to NanoString gold standard.

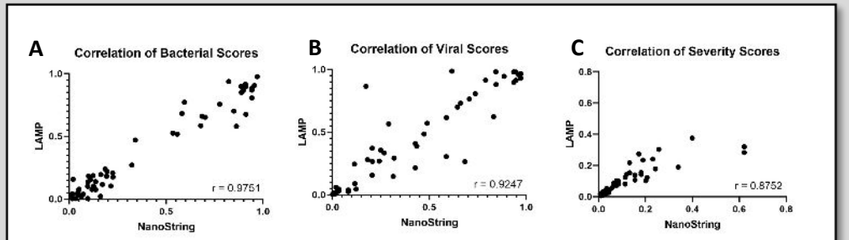


**Figure 2. The LAMP assay shows strong correlation with the nCounter gold standard.** Pearson correlation plot between LAMP and nCounter for (A) bacterial, (B) viral, and (C) severity scores. Pearson r values are 0.98, 0.93, and 0.88 respectively.

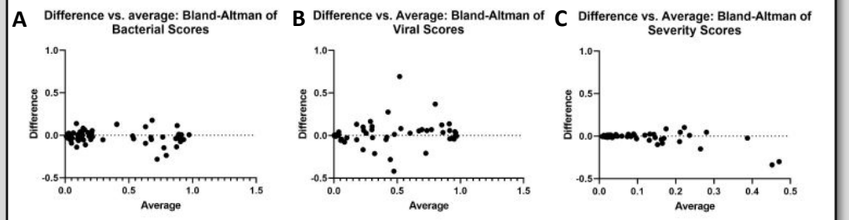


**Figure 3. Bland-Altman analysis provides additional evidence of strong agreement between LAMP and nCounter.** Bland-Altman plots of mean (average) vs. difference of scores between nCounter and LAMP approaches for the (A) bacterial score, (B) viral score, and (C) severity score. Bland-Altman analysis showed a 95% Limits of Agreement of -0.17 – 0.14, -0.26 – 0.30, and -0.15–0.12 for the bacterial, viral, and severity scores, respectively.

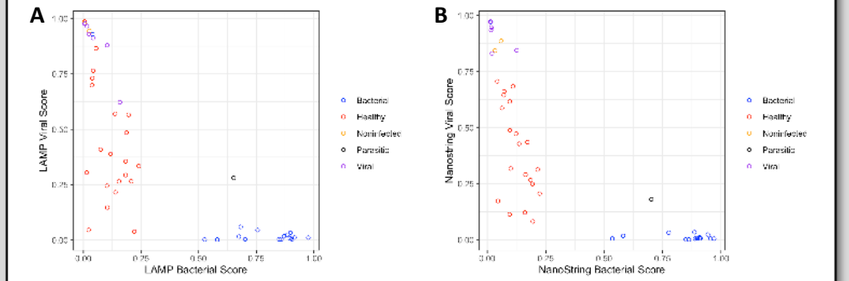


**Figure 4. InSep scores from both LAMP and nCounter are able to distinguish bacterial and viral infections.** Plots indicate distributions of bacterial and viral scores for (A) LAMP and (B) NanoString platforms among patients with known infection status ground truths.


**Figure 5. Prototype rendering of Myrna™.** Myrna is a point-of-care instrument being developed to quantify InSep's 29 mRNA's using LAMP with a turnaround time of <30 minutes. PAXgene whole blood tubes can be inserted into a microfluidic cartridge without need for pipetting, and <2 minutes of hands on time is required.

## Discussion

- **Findings:** Our LAMP assay demonstrated excellent accuracy for quantifying mRNAs against a gold standard of the NanoString nCounter platform using Pearson and Bland-Altman methods. Accuracy was high for samples from healthy volunteers but also those from subjects with defined bacterial and viral infections as well as severe infections, thereby allowing to distinguish bacterial from viral infections in patients presenting to the ED with suspected acute infections and sepsis.
- **Future Work:** Our rapid blood prep combined with LAMP advances the development of the InSep test to achieve a 30-minute turnaround time desired to optimally fit into the ED workflow for the assessment of patients with acute infection and sepsis. As a rapid (< 30-minute turnaround time) point-of-care solution suitable for the ED workflow. On a point-of-care platform, Myrna, InSep will be run in an automated fashion with minimal hands-on time (Figure 5).
- **Limitations:** This pilot analytical validity study was limited by the small sample size and selection of samples with high bacterial, viral, and/or severity scores. Extensive analytical validation work is ongoing to optimize our LAMP assay for transition onto the Myrna platform. Large analytical validity studies will also be performed to accommodate requirements for regulatory clearance in the US and countries accepting CE mark.
- **Clinical Implications:** When translated into a rapid point-of-care assay, the InSep test– based on it's high accuracy- has the potential to assist ED clinicians in making appropriate treatment decisions earlier, towards the ultimate goal of improving patient outcomes while achieving antimicrobial stewardship and conserving limited hospital resources.

## Acknowledgements

The authors wish to thank the patients who consented to participate in the study for their invaluable contributions to science.