out of the analysis. Still, the states included in our analyses are geographically representative and include more than 29 million children in the United States. As conversations around in-person education continue, hospitalization growth may offer reasons for concern.

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## Translation of a Host Blood RNA Signature Distinguishing Bacterial From Viral Infection Into a Platform Suitable for Development as a Point-of-Care Test

Discrimination of viral from bacterial infections remains a challenge, resulting in unnecessary investigation, admission, and antibiotic treatment of many patients with fever. Studies in *JAMA* previously reported that children with bacterial and viral infection can be distinguished by their blood host RNA signature.[1,2] Here, we demonstrate that a 2-gene RNA signature can be translated into a rapid (<25 minutes) and portable laboratory-on-a-chip platform suitable for development as a point-of-care test.

**Methods** | Herberg and colleagues[1,3] reported a 2-transcript signature (*IFI44L* and *FAM89A*) discovered using gene expression microarrays in a set of 455 children with fever and bacterial and viral infections (Inflammatory and Infectious Disease Study [IRIS] study). We randomly selected 24 RNA samples from patients in the IRIS study[1] with confirmed bacterial (n = 12) and viral (n = 12) infections, matched for severity, collected between September 2009 and May 2017, and extracted using the PAXgene Blood RNA Kit (PreAnalytiX GmbH). We identified transcripts suitable for translation to our laboratory-on-chip platform, which uses reverse transcription loop-mediated isothermal amplification (RT-LAMP), by assessing counts of *IFI44L* and *FAM89A* in a publicly available blood RNA sequencing data set comprising 255 children with bacterial and viral infections (GSE69529).[4] The average gene counts for *IFI44L* were sufficient (2281.9) but low for *FAM89A* (20.2), potentially compromising transferability across platforms. Therefore, using the list of previously identified 38 highly correlated transcripts,[1] we replaced *FAM89A* with *EMR1-ADGRE1* (RefSeq ID: NM_001974.5), which had sufficient average gene counts (511.3),[4] and in combination with *IFI44L* (RefSeq ID: NM_006820) had a similar performance to the original 2 transcript signature (area under the curve [AUC] in the training, test, and validation data sets: 93.4%, 97.4%, and 97.2%, respectively).

Our laboratory-on-chip platform combines novel pH-sensing complementary metal-oxide semiconductor technology[5] with RT-LAMP, which we term *electronic RT-LAMP* (RT-eLAMP) (**Figure 1**). RT-eLAMP uses thousands of microsensors (ion-sensitive field-effect transistors) to detect $H^+$ ions released, resulting in a change in pH, during the nucleic acid amplification process following the same experimental conditions as reported in our previous article.[6]

We compared the gene expression values (normalized $\log_2$ fluorescence for microarrays[1]) or time-to-positive values from signals of all the microsensors for RT-eLAMP using support vector classifiers, with 10-fold cross-validation, and each patient was assigned a score reflecting risk of bacterial or viral disease (package: scikit-learn in Python). We evaluated the predictive accuracy of the score in patients with microbiologically confirmed diagnoses using receiver operating characteristic curves, AUC,



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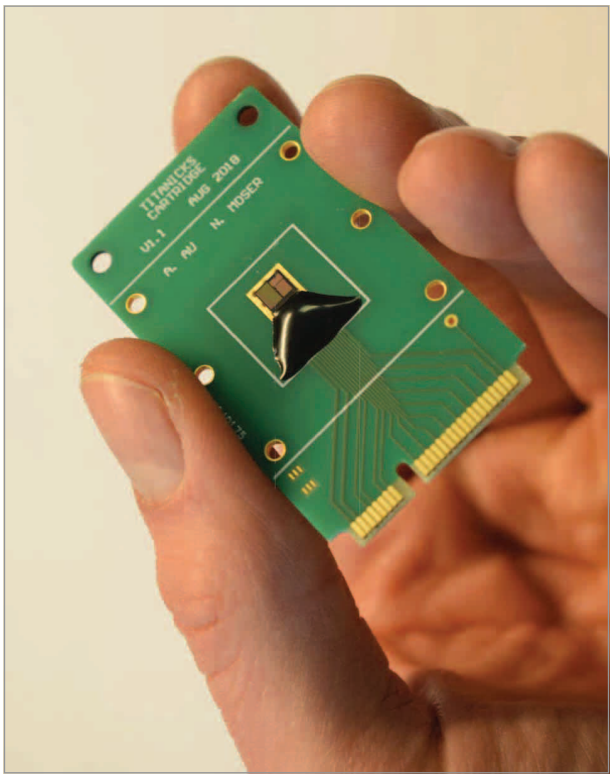
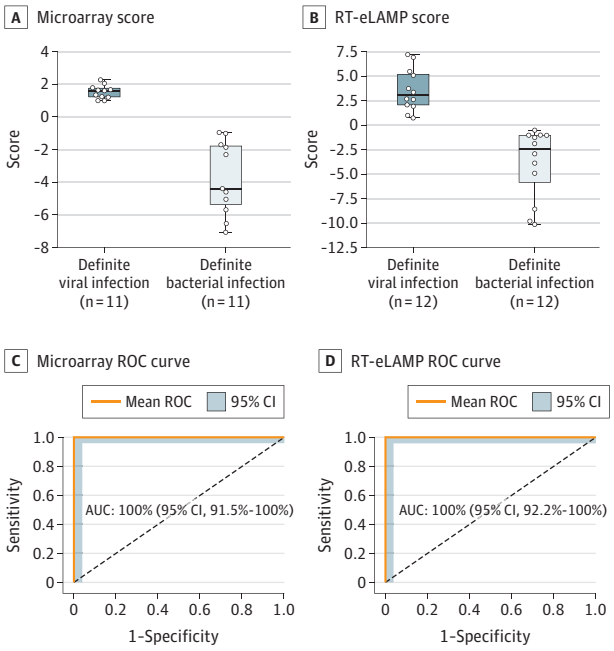
Figure 1. A Handheld Point-of-Care Test



Disposable diagnostic cartridge containing a complementary metal-oxide semiconductor ion-sensitive field-effect transistor array for on-chip real-time nucleic acid amplification.

Figure 2. Performance of the 2 Diagnostic Genes in Microarray and Electronic Reverse Transcription Loop-Mediated Isothermal Amplification (RT-eLAMP)




Predicitive score, receiver operating characteristic (ROC) curves, and expression values based on the 2-transcript signature for distinguishing definite viral (dark blue) and definite bacterial (light blue) samples. Boxes indicate the interquartile ranges and the median (middle line). Whiskers indicate 1.5 times the interquartile range. A and C, Performance using expression values obtained from the microarray platform. B and D, Performance using our RT-eLAMP platform. AUC indicates area under the curve.

and 95% CIs under the binomial distribution. Analysis began April 2020.

**Results** | Using microarray data,[1] the 2-transcript signature applied to children with bacterial and viral infections had a sensitivity and specificity of 100% (95% CI, 95.7%-100%) and 100% (95% CI, 95.7%-100%), respectively, with AUC of 100% (95% CI, 91.5%-100%) (**Figure 2**A and B). Translating the 2-gene signature to RT-eLAMP showed similar results: sensitivity and specificity were 100% (95% CI, 96.0%-100%) and 100% (95% CI, 96.0%-100%), respectively, with AUC of 100% (95% CI, 92.2%-100%) (Figure 2C and D). The calculated limit of detection for the 2 assays were 10 RNA copies per reaction in 10-µL reaction volume and time to positive under 25 minutes, much faster than microarray.

**Discussion** | Previously reported RNA signatures that differentiate bacterial from viral infection were discovered using cumbersome transcriptomic technologies. We have moved the promising transcript signatures closer to clinical application by establishing that the 2-transcript signature can be detected using a semiconductor-based sensing platform combined with isothermal amplification chemistries. The absence of fluorescent labels and the economy of scale of the microchip industry makes the technology potentially suitable for implementation at low cost (<£1 [US $1.33] per chip). While our study includes modest patient numbers, it provides a proof of concept that host RNA signatures can be detected rapidly and cost-effectively in a format suitable for development as a point-of-care diagnostic test that might be applied to a range of clinical diagnoses. This study has a number of limitations including a relatively small sample size, a focus on confirmed infections, and that the platform currently lacks integrated sample preparation and has a limited numbers of wells per microchip.

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## Solitary Use of Alcohol and Marijuana by US 12th Grade Students, 1976-2019

Recent reviews have highlighted adolescent solitary alcohol and marijuana use as risk indicators associated with negative consequences, coping motives, and negative affect[1,2]; solitary use may reflect self-medication.[1,2] Adolescent solitary alcohol use is associated with health and academic problems,[3] deviant behavior,[3] and alcohol use disorder.[4] Data on sex differences in solitary alcohol and marijuana use have been mixed.[1,2] Nationally representative estimates of prevalence and change in adolescent solitary alcohol and marijuana use are needed.[1] This study provides 2018-2019 prevalence estimates of and 1976-2019 trends in solitary alcohol and marijuana use among all 12th grade students and those who used alcohol and marijuana in the past 12 months, separated by sex.

**Methods** | Data were collected from spring 1976 through spring 2019 through the US nationally representative Monitoring the Future study.[5] Informed consent (active or passive, per school policy) was obtained from parents (if students were <18 years) or students (if students were ≥18 years). A University of Michigan institutional review board approved the study. Mean student response rates were 82.4%.[5] Solitary use was asked on 1 of 6 randomly distributed questionnaires. Respondents self-reported past 12-month alcohol and marijuana use and how often such use occurred when alone. For each substance, a dichotomous measure was coded, indicating any solitary use. Prevalence and SE estimates (calculating means of 2-year groupings to enhance stability) were obtained using survey procedures in SAS 9.4 (SAS Institute Inc), accounting for the complex sample design. Using obtained estimates, models[6] estimated linear change over time using Joinpoint version 4.7.0.0 (National Cancer Institute); statistical significance was defined as $P < .05$ (2 sided).

**Results** | Of the 121 279 students responding to the relevant questionnaire, 9171 (7.6%) had missing data on sex; another 1482 (1.2%) had missing data on past-12-month alcohol and/or marijuana use or solitary use, leaving 110 626 responses. The sample included 57 257 female students (51.8%). Among all 12th grade students in 2018-2019, 14.8% (95% CI, 13.4%-16.3%) reported solitary alcohol use and 15.8% (95% CI, 14.2%-17.4%) reported solitary marijuana use. Among those who had used alcohol in the past 12 months in 2018-2019, solitary drinking was reported by 23.5% (95% CI, 20.4%-26.6%) of female students and 30.0% (95% CI, 26.1%-33.9%) of male students; percentages for solitary marijuana use among those who used marijuana were 42.3% (95% CI, 37.7%-47.0%) of female students and 54.8% (95% CI, 50.0%-59.6%) of male students.

Trend analyses (**Figure**; **Table**) showed among all students, solitary alcohol use decreased nonsignificantly from 1976-1977 through 1986-1987 (slope 1 [SE], −0.547 [0.254]; $P = .05$), showed a slope not significantly different than 0 through 1992-1993, and then decreased significantly through 2018-2019 (slope 3 [SE], −0.182 [0.060]; $P = .009$). Solitary marijuana use among all students decreased signifi-



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