Diagnostic Accuracy of an At-Home, Rapid Self-Test for Influenza

Rachel E. Geyer, Jack Henry Kotnik, Victoria Lyon, Elisabeth Brandstetter, Monica Zigman Suchsland, Peter D. Han, Chelsey Graham, Misja Ilcisin, Ashley E. Kim, Helen Y. Chu, Deborah A. Nickerson, Lea M. Starita, Trevor Bedford, Barry Lutz, Matthew J. Thompson

Submitted to: JMIR Public Health and Surveillance
on: March 05, 2021

Table of Contents

Original Manuscript **5**
Supplementary Files **25**
Figures **26**
Figure 1 ..... **27**
Multimedia Appendixes **28**
Multimedia Appendix 1 ..... **29**
Multimedia Appendix 2 ..... **29**
Multimedia Appendix 3 ..... **29**
Multimedia Appendix 4 ..... **29**
Multimedia Appendix 5 ..... **29**

Diagnostic Accuracy of an At-Home, Rapid Self-Test for Influenza

Rachel E. Geyer[1*] MPH; Jack Henry Kotnik[1, 2*] BA; Victoria Lyon[1] MPH; Elisabeth Brandstetter[3, 4] MPH; Monica Zigman Suchsland[1] MPH; Peter D. Han[4, 5] MS; Chelsey Graham[6] MEng; Misja Ilcisin[7] BS; Ashley E. Kim[3] BS; Helen Y. Chu[3, 4] MD; Deborah A. Nickerson[4, 5] PhD; Lea M. Starita[4, 5] PhD; Trevor Bedford[4, 5, 7] PhD; Barry Lutz[2, 4] PhD; Matthew J. Thompson[1] MD, MPH, DPhil

[1]Department of Family Medicine, University of Washington Seattle US
[2]Department of Bioengineering, University of Washington Seattle US
[3]Department of Medicine, University of Washington Seattle US
[4]Brotman Baty Institute, University of Washington Seattle US
[5]Department of Genome Sciences, University of Washington Seattle US
[6]Massachusetts Institute of Technology Cambridge US
[7]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center Seattle US
[*]these authors contributed equally

Corresponding Author:
Rachel E. Geyer MPH
Department of Family Medicine, University of Washington
4225 Roosevelt Way NE Suite 308
Seattle
US

Abstract

Background: Rapid diagnostic tests (RDT) for influenza used by individuals at home could potentially expand access to testing and reduce the impact of influenza on health systems. Improving access to testing could lead to earlier diagnosis following symptom onset, allowing more rapid interventions on those who test positive, including behavioral changes to minimize spread. However, the accuracy of RDTs for influenza has not been determined among self-testing populations.

Objective: To assess the accuracy of an influenza RDT conducted at home by lay users with acute respiratory illness (ARI), compared to a self-collected sample conducted by the same individual mailed to a laboratory for reference testing.

Methods: A comparative accuracy study of an at-home influenza RDT (Ellume, Brisbane, Qld, Australia) in a convenience sample of individuals experiencing ARI symptoms. Participants were enrolled in February and March 2020, from the greater Seattle region, Washington, USA. Participants were mailed the influenza RDT and reference sample collection materials, which they completed and returned for RT-qPCR influenza testing in a central laboratory. We explored the impact of age, influenza type, duration, and severity of symptoms on RDT accuracy, viral CT (cycle threshold), and a marker of human DNA (RNase P).

Results: 605 participants completed all study steps and were included in our analysis, of whom 87 (14.4%) tested positive for influenza by RT-qPCR (70 influenza A, 17 influenza B). The overall sensitivity and specificity of the RDT compared to the reference test were 61% (95%CI 50-71) and 95% (95%CI 93-97), respectively. Among individuals with symptom onset ?72 hours, sensitivity was 63% (95%CI 48-76) and specificity was 94% (95% CI 91-97), while for those with duration > 72 hours, sensitivity and specificity were 58% (95%CI 41-74) and 96% (95%CI 93-98), respectively. Viral load on reference swabs was negatively correlated with symptom onset, while quantities of the endogenous marker gene RNase P did not differ between PCR positive/negative groups, age groups, or influenza subtypes. The RDT did not have higher sensitivity or specificity among those who reported more severe illness.

Conclusions: The sensitivity and specificity of the self-test was comparable to that of influenza RDTs used in clinical settings. False negative self-test results were more common when the test was used after 72 hours of symptom onset, but were not related to inadequate swab collection, or severity of illness. Deployment of home tests may provide a valuable tool to support management of influenza and other respiratory infections.

(JMIR Preprints 05/03/2021:28268)
DOI: https://doi.org/10.2196/preprints.28268

Preprint Settings

1) Would you like to publish your submitted manuscript as preprint?

✓ **Please make my preprint PDF available to anyone at any time (recommended).**
Please make my preprint PDF available only to logged-in users; I understand that my title and abstract will remain visible to all users.
Only make the preprint title and abstract visible.
No, I do not wish to publish my submitted manuscript as a preprint.

2) If accepted for publication in a JMIR journal, would you like the PDF to be visible to the public?

✓ **Yes, please make my accepted manuscript PDF available to anyone at any time (Recommended).**
Yes, but please make my accepted manuscript PDF available only to logged-in users; I understand that the title and abstract will remain v
Yes, but only make the title and abstract visible (see Important note, above). I understand that if I later pay to participate in <a href="http

Original Manuscript

TITLE: Diagnostic Accuracy of an At-Home, Rapid Self-Test for Influenza

Authorship: Rachel E. Geyer, MPH[1]*, Jack Henry Kotnik, BA[1,2]*, Victoria Lyon, MPH[1], Elisabeth Brandstetter, MPH[3,4], Monica Zigman Suchsland, MPH[1], Peter D. Han MS[4,6], Chelsey Graham, MEng[7], Misja Ilcisin, BS[5], Ashley E. Kim, BS[3], Helen Y. Chu, MD[3,4], Deborah A. Nickerson, Ph.D.[4,6], Lea M. Starita, PhD[4,6], Trevor Bedford, PhD[4,5,6], Barry Lutz, PhD[2,4], Matthew J. Thompson, MD, MPH, DPhil[1] , on behalf of the SFS investigators.

Author Affiliations:

[1]Department of Family Medicine, University of Washington
[2]Department of Bioengineering, University of Washington
[3]Department of Medicine, University of Washington
[4]Brotman Baty Institute, University of Washington
[5]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center
[6]Department of Genome Sciences, University of Washington
7Massachusetts Institute of Technology
*these authors contributed equally

Introduction

In the most recent influenza season in the United States (October 2019 to April 2020), an estimated 39-62 million people were infected, resulting in 18-26 million health care visits and 24,000-62,000 deaths [1]. The economic impacts are proportional - the 2018 seasonal influenza cost the United States an estimated $11.2 billion, including $3.2 billion in direct medical costs and an estimated 20.1 million productive hours lost [2]. Negative impacts on health and the economy may be improved by early intervention to diagnose those with influenza and intervene with antiviral treatment and/or behavioral changes to reduce transmission.

Diagnosis of influenza based on clinical features alone is inaccurate; therefore, several clinical guidelines support laboratory testing of respiratory tract specimens (usually nasal or nasopharyngeal) to detect influenza virus. Increasingly, laboratory testing for influenza has shifted to in-clinic testing using point-of-care (POC) devices [3]. Rapid diagnostic tests (RDT) are a class of POC tests that can be performed with a few simple steps and typically do not require instrumentation or special supplies, raising the possibility for untrained individuals to use these tests outside of clinical settings [4]. Influenza RDTs for home use could potentially expand access to testing and lower costs, thus facilitating earlier diagnosis and reducing the time from symptom onset to appropriate care, such as receiving antiviral treatment or making behavioral changes to minimize spread [5]. Advantages of home testing for influenza and other respiratory viruses could be even more critical in pandemic situations, where isolating cases and limiting contact with potential cases are essential components of containing outbreaks [6,7].

Several studies have already investigated the accuracy of self-swabbing and self-testing for influenza. A recent systematic review of 13 studies found that influenza was detected by self-collected nasal or mid-turbinate samples, with similar accuracy to samples collected by healthcare professionals [8]. RDTs tested in routine healthcare settings have shown sensitivities and specificities of 60%-70% and 90-100% respectively [9, 10], but due to the novelty of home-testing, few RDTs have been studied in the home environment. There are currently no FDA tests approved for detection of influenza at home.

A primary hurdle to at-home testing for influenza or other respiratory viruses is that RDTs are typically less accurate than laboratory-based assays, even when used by healthcare workers [11,12]. Numerous variables impact performance of the test, including quality of the sample, infection prevalence, timely testing after illness onset, and lower viral load in less severe cases. These variables have not been well-studied in POC settings [9,13–15] nor in at-home populations; to our knowledge, only one publicly available study (a work in progress from our group) has attempted to assess the accuracy and feasibility of performing an entire self-test at home using an RDT [16].

In this study, we assess the accuracy of an influenza RDT conducted at home by lay users with influenza-like-illness, compared to a self-collected sample conducted by the same individual mailed to a laboratory for reference testing.

Methods

Study Design

We conducted a prospective, comparative accuracy study of an at-home influenza RDT in a convenience sample of individuals experiencing acute respiratory illness (ARI). The study was conducted as a sub-study within the Seattle Flu Study (SFS) which has conducted city-wide community surveillance for influenza and other respiratory viral infections. The SFS involved same-day self-swab samples [17]; participants who qualified and enrolled in the self-test sub-study reported here received an additional at-home influenza RDT. The RDT results were compared to the results of a self-collected mid-turbinate nasal swab sample returned by mail and tested by a laboratory RT-qPCR (quantitative reverse-transcription polymerase chain reaction) assay as described below [17]. Participants also answered a questionnaire that included information about their symptoms, risk factors, and demographics. The study was approved by the University of Washington Human Subjects Division (STUDY00006181). Reporting of this study adheres to

Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidance [18].

Recruitment

Participants were recruited using targeted online advertisements on websites, including Facebook, Instagram, Twitter, and Google. Additional recruitment occurred through in-person referrals from community kiosks set up by the SFS, healthcare providers, travel clinics, immigrant/refugee health screenings, local schools, and workplaces. Potential subjects were directed to a study website, where they were screened for eligibility based on age, zip code, symptoms, time from symptom onset, and their smartphone operating system.

Sample Size

The recruitment goal was to enroll 3,000 participants into the study, assuming 80% (2,400) of participants would complete all steps and assuming an influenza prevalence rate of 12.5%, which would provide 300 influenza-positive samples. Following the study's completion, electronic gift cards of up to $20 were sent to all participants.

Participants

Participants were enrolled from February 19th to March 9th, 2020, from the greater Seattle, Washington, USA area, a population of 744,000. Eligibility criteria included those who self-identified as having a cough or at least two new or worsening ARI symptoms (i.e., feeling feverish, headache, chills or shivering, sore throat, nausea or vomiting, runny or stuffy nose, malaise, muscle or body aches, trouble breathing, diarrhea, rash, ear pain or discharge) in the previous 72 hours [19,20]. Additionally, participants were required to be 5 or more years of age, residing or working within a list of eligible zip codes, able to understand study instructions in English, and able to conduct testing using a Bluetooth-enabled device.

Pre-Test Data Collection

Participants consented electronically; a parent or legal guardian consented for participants under 18 years of age, and assent forms were provided for participants 13 to 18 years of age using Research Electronic Data Capture (REDCap)[21], hosted at the University of Washington Institute of Translational Health Sciences. After consenting, the REDCap instrument obtained the participant's home address and contact information to allow the delivery of a Flu Kit by courier.

Flu Kit Components and Delivery

Flu Kits were fabricated by the study team to comply with US regulations for shipping Biological Substance (Category B) [22]. The Flu Kit contained one instructional quick start guide, one influenza RDT labeled as a research device (Ellume Home Flu Test (EFHT), Brisbane, Qld, Australia)) which included one mid-turbinate swab, buffer fluid, dropper, and Bluetooth-enabled sample analyzer, and a reference sample kit containing one mid-turbinate swab (Copan, Murrieta, CA, USA FLOQSwabs® 56380CS01), one tube with 3mL of viral transport medium (VTM) (BD, Franklin Lakes, NJ, USA, cat. #220220), one specimen transport bag with absorbent sleeve (VWR, Radnor, PA, USA, cat. #11215-684), one return box (ULINE, Pleasant Prairie, WI, USA, S-16524), and one return mailer (ULINE, S-3355) overpack. If participants reported errors with the EHFT, a second kit was sent out as soon as possible during staffing hours (within 12 hours).

A Flu Kit was sent to participants' homes within 24 hours of their enrollment, with the majority sent within two hours. Enrollments that were received after business hours were processed and mailed the following morning. Each kit included a unique barcode number (located in three places in each kit: on the 3mL tube, return mailer, and quick start guide) to link surveys, EHFT results, and reference test results for analysis. Once participants completed their at-home study procedures, they were instructed to mail their reference sample, using the materials provided, to the University of Washington research laboratory via the US Postal Service within 24 hours of completing their test.

At-home test and data collection

Upon receiving their Flu Kit at home, participants were instructed to complete a questionnaire on REDCap (sent via email). This included questions about their symptoms and exposure risks, including housing, health conditions, recent travel, and demographics (Multimedia Appendix 1).

Participants were instructed to download the EHFT app onto their Bluetooth-enabled device. The app provided an instructional video, followed by step-by-step on-screen instructions for sample self-collection using the included custom mid-turbinate swab. Participants were instructed to insert and rotate the swab three times around their nasal cavity on both nostrils. They then placed the swab sample in the buffer, added this buffer fluid to the analyzer, and waited 12 minutes to process the sample. The analyzer then sent test results directly to the EHFT app on the user's device via Bluetooth and a secure research database. Because participants were using an experimental research device, they were blinded to the EHFT test results. Participants received a "thank you" screen in the EHFT app once their sample was processed that instructed them to refer to study instructions for completing their reference sample and contact their healthcare provider if they were concerned about their symptoms.

Participants were also asked to obtain a second mid-turbinate swab using the swab included in their Flu Kit, following written and photo instructions on both the quick start guide and REDCap survey (Multimedia Appendix 1). They were instructed to insert the swab halfway (about 1 inch) into either nostril and press against the side and rotate five times. They were then instructed to place the swab into the collection tube, repackage all components to meet US regulations for shipping biological substances (UN3373 Category B) [23] and return via the US Postal Service to the UW research lab.

Participants received a follow-up survey seven days following enrollment, which included questions about illness duration and severity, recent travel, and feedback for the research team (Multimedia Appendix 1).

Reference Testing

Returned kits received in the laboratory were examined, and any evidence of damage to sample or packaging was documented. Samples were split into two 1mL aliquots. One aliquot was frozen at -80℃, and the other was stored at 4℃ until extraction. All samples were run in duplicate. 200µL of VTM were extracted using Magna Pure 96 small-volume total nucleic acids extraction kit (Roche, Basel, CH, Prod. #06543588001). Purified total nucleic acids were tested against a panel of respiratory pathogens using the Taqman OpenArray platform (Thermofisher, Waltham, MA, USA) for RT-qPCR. The OpenArray panel included probe sequences for influenza A H3N2, influenza A H1N1, and pan influenza A, influenza B, influenza C, respiratory syncytial viruses (RSV) A and B, human coronaviruses (hCoV) 229E, NL63, OC43, and HKU1, adenovirus (AdV), human rhinovirus (hRV), human metapneumovirus (hMPV), human parechovirus (hPeV), enteroviruses A, B, C, D, D68, and G, human bocavirus (hBoV), *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. The OpenArray panel also included probes for the human gene Ribonuclease P (RNase P) as an indicator of sample quality. All quantitative data were captured as Relative Threshold Cycles (Crt), which is approximately 10 cycles less than an equivalent qPCR cycle threshold (Ct).

All EHFT and laboratory reference test results were linked to participants through their unique barcode. Laboratory personnel did not have access to EHFT results or clinical information when interpreting the reference assay. A reference test was considered positive for a pathogen if RT-qPCR generated a fluorescent signal for the channel-specific pathogen within 40 PCR cycles. EHFT or laboratory results were not visible to participants.

Data Analysis

A participant flow diagram was created demonstrating each major step in the study and participant dropout. Summary statistics were calculated for participant demographics, risk factors, ARI symptoms, symptom severity, and other pathogens detected. Pearson's Chi-squared test with Yates' continuity correction was calculated for risk factors, symptom presence, and symptom severity between reference test positive (PCR positive) and negative (PCR negative) participants. P-values <0.05 were considered statistically significant. We calculated symptom onset as the difference between the self-reported symptom onset date and the exact time when the EHFT was completed. Participants were instructed to collect the reference swab immediately after taking the EHFT.

We calculated sensitivity, specificity, and positive and negative likelihood ratios (with 95% confidence intervals (CI)) for the overall performance of the index test (EHFT) compared to the reference test (OpenArray RT-qPCR), and independently for influenza A and B. In addition, we analyzed data by subgroups that previously have been shown to impact viral load [24–26], namely symptom onset prior to testing, and illness severity measured as total number of symptoms (1 to 9 symptoms) and disruption of daily life caused by their illness (1-5 scale, 1 being not at all, 5 being very much). For each subgroup, we calculated sensitivity, specificity, and positive and negative likelihood ratios with 95% CI. We performed pairwise comparisons of the mean level of impact on activities between subgroups using One-Way ANOVA and Tukey's Honestly Significant Difference (HSD) Test. Where appropriate, Pearson's correlations were calculated.

The average influenza Crt value was used as a proxy for relative viral load; lower Crt values correspond to higher viral loads and thus fewer cycles to generate a sufficient OpenArray signal [27]. Each additional Crt cycle is equivalent to a roughly 2-fold reduction in genomic copies of viral RNA. Means and standard deviations were calculated for the following subgroups, symptom onset, influenza subtype, child/adult (5-17 years versus 18 and older), and true-positive versus false-negative subgroups. Pairwise comparisons of mean influenza Crt for the subgroups were performed using Student's t-test. Multiple linear regression models were fit separately for average Crt as a function of symptom onset, adjusted for age, and number of symptoms and level of impact on daily activities, both adjusted for age and symptom onset.

The RNase P Crt value was used as an indicator of sample quality; lower Crt corresponds to more endogenous human DNA in the sample, indicating greater likelihood of sufficient material collected on the swab [25, 28, 29]. Median RNase P Crt values were compared between age groups, and between true-positive (TP), false-positive (FP), false-negative (FN), and true-negative (TN) test result subgroups using a Kruskal-Wallis test on ranks and Dunn's multiple comparisons post-hoc test. Median RNase P Crt values were compared between PCR positive and PCR negative groups, influenza A and B positive groups, and between child (≤18) and adult (>18) groups using a Mann-Whitney U test.

Participants with missing or indeterminate EHFT or reference samples were removed from the analysis. The analysis was conducted in R (Version 1.3.1056) [30].

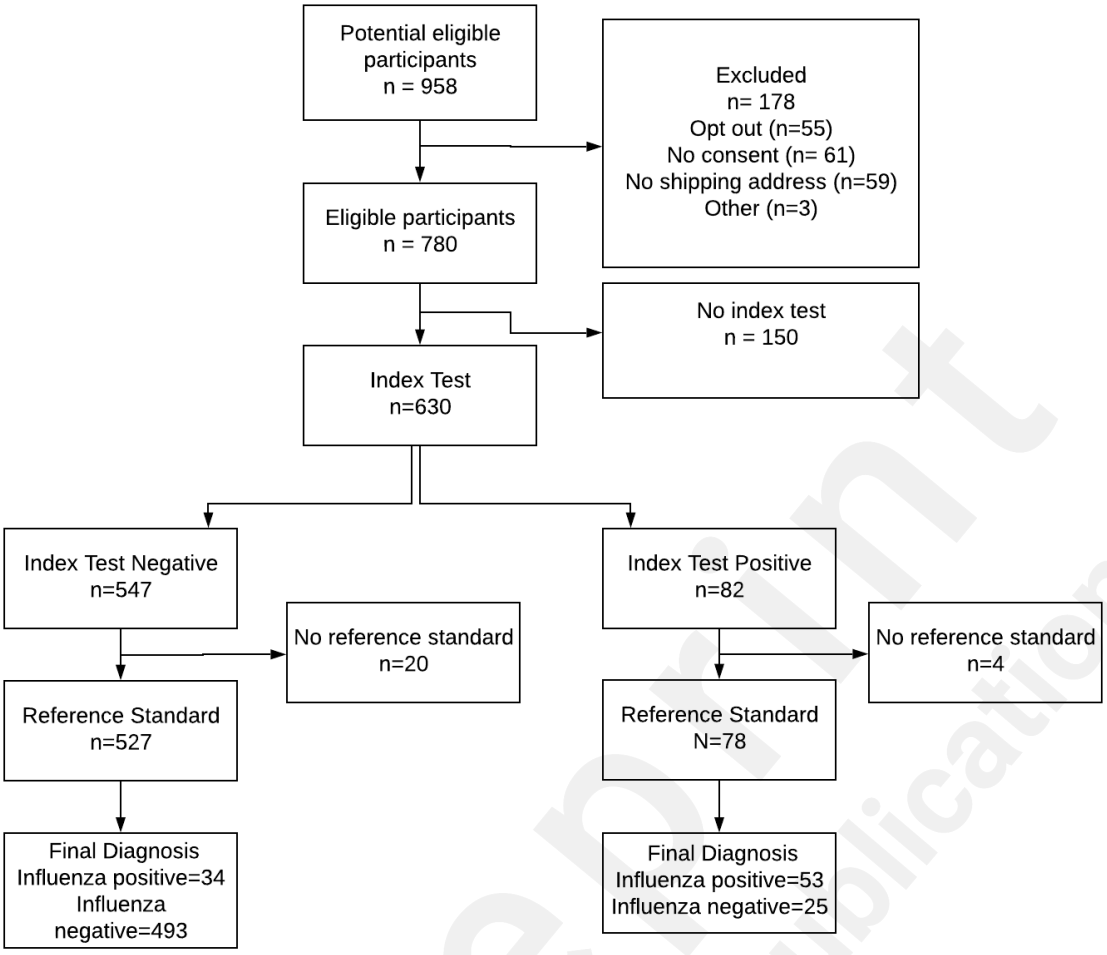
Funding
The Seattle Flu Study was funded by Gates Ventures. The funder was not involved in the design of the study, does not have any ownership over the management and conduct of the study, the data, or the rights to publish.

Results

Participant Recruitment and Retention

A total of 958 participants met inclusion criteria (Figure 1), of whom 780 (81.4%) completed the consent form, provided a viable shipping address, and were sent a Flu Kit. Of those who received their kit, 630 (80.8%) completed the index test. One individual who completed their index test 34 days after symptom onset was excluded. Of those that completed the index test 605 (96.0%) also returned the reference sample to the lab and are included in our analysis. The final study sample included in this analysis were the 605 participants who completed both the index and reference tests.

Figure 1. Recruitment flow for the Home Flu Test participants



The majority of participants were recruited through advertisements on Facebook, Instagram or Twitter (184, 30.4%); friend or family referrals (181, 29.9%); and other online media (138, 22.8%). Google ads and healthcare provider referrals recruited more influenza positive participants than other recruitment methods.

Participant Demographics

Most participants were 25-44 years of age (63.0%), female (59.4%), white (71.0%), and had completed a bachelor's (38.6%) or advanced (38.9%) degree (Table 1). The majority had private health insurance (85.8%), received the 2019 influenza vaccination (70.0%), and were non-smokers (85.3%). Age (P = 0.017) and education (P = 0.017) were significantly associated with a positive influenza PCR test, but receipt of the 2019/2020 influenza vaccine was not associated with positive influenza PCR test. The most frequent symptoms reported were fatigue (72.4%), sore throat (68.4%), cough (58.7%), headache (56.5%), and fever (48.3%)(Table 2). Fever, cough, chills, sweats, nausea or vomiting, myalgia (all P < .001), fatigue (P = .007), runny nose (P = .037), and difficulty breathing (P = .047) were all significantly associated with a positive PCR test. Participants with positive influenza PCR were also more likely to report moderate or severe levels of fever (P < .001), cough (P < .001), fatigue (P < .001), myalgia (P < .001), and sore throat (P = .037) compared to those with a negative PCR test. The Taqman OpenArray identified 186 participants who tested positive for one or more respiratory pathogens other than influenza, some of which were co-infections with influenza A or B (Multimedia Appendix 2). Other than influenza, the most common respiratory pathogens identified were rhinovirus (77) and seasonal human coronavirus (50).

Table 1: Characteristics of participants with and without influenza detected on reference test

(N=605)

		Overall (N=605)	Influenza PCR positive (n=87)	Influenza PCR negative (n=518)	
Demographics		N (%)	n (%)	n (%)	***P* value**
Age (years)					.017
	5-12	29 (4.8)	10 (11.5)	19 (3.7)	
	13-17	4 (0.7)	1 (1.1)	3 (0.6)	
	18-24	54 (8.9)	6 (6.9)	48 (9.3)	
	25-34	222 (36.8)	23 (26.4)	199 (38.4)	
	35-44	159 (26.2)	26 (29.9)	133 (25.7)	
	45-64	112 (18.5)	18 (20.7)	94 (18.1)	
	65+	12 (2.0)	1 (1.1)	11 (2.1)	
Gender					.065
	Male	217 (36.0)	35 (40.2)	182 (35.1)	
	Female	360 (59.4)	47 (54.0)	313 (60.4)	
	Other	4 (0.7)	2 (2.3)	2 (0.4)	
Education					.017
	Less than high school	3 (0.5)	2 (2.3)	1 (0.2)	
	Graduated high school/GED**	15 (2.5)	0 (0.0)	15 (2.9)	
	Some college	55 (9.2)	5 (5.7)	50 (9.7)	
	Bachelor's degree	234 (38.6)	37 (42.5)	197 (38.0)	
	Advanced degree	236 (38.9)	28 (32.2)	208 (40.2)	
Race*					
	White	430 (71.0)	69 (79.3)	361 (69.7)	.107
	Black	9 (1.5)	3 (3.4)	6 (1.2)	.251
	Asian	137 (22.8)	10 (11.5)	127 (24.5)	.010
	AI, AN, NH, PI***	10 (1.7)	0 (0.0)	10 (1.9)	N/A
	Other	21 (3.5)	3 (3.4)	18 (3.5)	1.00
Hispanic	Yes	31 (5.1)	2 (2.3)	29 (5.6)	.314
Health Insurance*					
	Public	42 (6.9)	4 (4.6)	38 (7.3)	.477
	Private	519 (85.8)	76 (87.4)	443 (85.5)	.909
	Other	7 (1.2)	1 (1.1)	6 (1.2)	1.00
	No insurance	11 (1.8)	3 (3.4)	8 (1.5)	.429
2019-2020 Influenza vaccination	Yes	424 (70.0)	62 (71.3)	362 (69.9)	.784
Current smoker	No	516 (85.3)	71 (81.6)	445 (85.9)	.442

*Totals may exceed 100% because participants could select multiple responses
**GED: General Education Development

***American Indian, Alaskan Native, Native Hawaiian, or Pacific Islander

Table 2. Presence and severity of symptoms reported by participants with and without influenza detected on reference test

	Overall (N=605)	**Influenza PCR positive (n=87)**	**Influenza PCR negative (n=518)**	
Symptoms	N (%)	n (%)	n (%)	***P* value**
*Feeling feverish**	292 (48.3)	76 (87.4)	216 (41.7)	< .001
Mild	119 (19.7)	14 (16.1)	105 (20.3)	< .001
Moderate	122 (20.2)	34 (39.1)	88 (17.0)	
Severe	51 (8.4)	28 (32.2)	23 (4.4)	
Headache	342 (56.5)	63 (72.4)	279 (53.9)	.002
Cough*	355 (58.7)	72 (82.8)	283 (54.6)	<.001
Mild	168 (27.8)	17 (19.5)	151 (29.2)	<.001
Moderate	150 (24.8)	37 (42.5)	113 (21.8)	
Severe	36 (6.0)	18 (20.7)	18 (3.5)	
Chills or shivering	227 (37.5)	68 (78.2)	159 (30.7)	< .001
Sweats	142 (23.5)	43 (49.4)	99 (19.1)	< .001
Sore throat or itchy/scratchy throat*	414 (68.4)	57 (65.5)	357 (68.9)	.53
Mild	174 (28.8)	27 (31.0)	147 (28.4)	.037
Moderate	189 (31.2)	18 (20.7)	171 (33.0)	
Severe	49 (8.1)	11 (12.6)	38 (7.3)	
Nausea or vomiting	72 (11.9)	21 (24.1)	51 (9.8)	< .001
Running or stuffy nose	358 (59.2)	61 (70.1)	297 (57.3)	.037
Feeling more tired than usual*	438 (72.4)	74 (85.1)	364 (70.3)	.007
Mild	105 (17.4)	11 (12.6)	94 (18.1)	< .001
Moderate	217 (35.9)	27 (31.0)	190 (36.7)	
Severe	116 (19.2)	36 (41.4)	80 (15.4)	
Muscle or body aches*	300 (49.6)	65 (74.7)	235 (45.4)	< .001
Mild	99 (16.4)	4 (4.6)	95 (18.3)	< .001
Moderate	146 (24.1)	37 (42.5)	109 (21.0)	
Severe	55 (9.1)	24 (27.6)	31 (6.0)	
Increased trouble with breathing	134 (22.1)	27 (31.0)	107 (20.7)	.047
Diarrhea**	69 (11.4)	6 (6.9)	63 (12.2)	.19
Rash**	9 (1.5)	0 (0.0)	9 (1.7)	.44

Ear pain or discharge**	54 (8.9)	11 (12.6)	43 (8.3)	.31

*Included survey questions on severity for a subset of symptoms which are presented in this table
**Symptoms reported for children (<18 years) only

Almost all (99.8%) participants completed the EHFT within 15 days of symptoms onset (Range: 0.6 - 14.4 days) (Multimedia Appendix 2), with average time from symptom onset to EHFT testing of 2.9 days (SD = 1.5 days). A total of 344 (56.9%) participants took the EHFT within 72 hours (3 days) after symptom onset, 249 (41.2%) between 4 and 7 days, and 12 (1.9%) between 8 and 15 days after symptom onset. The longest median time interval segment between symptom onset and EHFT testing was between symptom onset and study enrollment (median 48 hours), compared to time from enrollment to kit shipping (median 1.25 hours), and from kit shipping to testing (median 5.33 hours).

Accuracy of RDT

A total of 87 (14.4%) participants tested positive for influenza by the reference test (70 influenza A, 17 influenza B). The overall sensitivity and specificity of the EHFT compared to the reference test was 61% (95%CI 50-71) and 95% (95%CI 93-97), respectively (Table 3: Accuracy). The sensitivity and specificity of the EHFT for influenza A were 60% (95%CI 48-72) and 99% (95%CI 98-100) respectively, and for influenza B were 65% (95%CI 38-86) and 96% (95%CI 94-98) respectively. Influenza B yielded a higher rate of false-positive tests, which resulted in a positive predictive value (PPV) of 33% (95%CI 18-52).

Subgroup analysis of EHFT accuracy based on time from symptom onset to conducting the EHFT (Table 3) found sensitivity and specificity of influenza A detection at ≤72 hours of symptom onset were 62.5% (95%CI 48-77) and 100% (95% CI 98-100), respectively, while at >72 hours they were 57% (95%CI 37-75) and 99% (95%CI 97-100), respectively. The sensitivity and specificity of influenza B at ≤72 hours were 64% (95%CI 31-89) and 95% (95%CI 92-97) respectively, while at >72 hours they were 67% (95%CI 22-96) and 98% (95%CI 95-99), respectively.

Table 3: Accuracy of Ellume home influenza test compared to laboratory reference PCR

	True Positive	False Positive	False Negative	True Negative	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
Overall (N)								
All (605)	**53**	**25**	**34**	**493**	**61% (50-71)**	**95% (93-97)**	**68% (56-78)**	**94% (91-95)**
≤ 72 Hours (344)	32	17	19	276	63% (48-76)	94% (91-97)	65% (50-78)	94% (90-96)
> 72 Hours (261)	21	8	15	217	58% (41-74)	96% (93-98)	72% (53-87)	94% (90-96)
Influenza A								
All (605)	**42**	**3**	**28**	**532**	**60% (48-72)**	**99% (98-100)**	**93% (82-99)**	**95% (93-97)**
≤ 72 Hours (344)	25	1	15	303	63% (46-77)	100% (98-100)	96% (80-100)	95% (92-97)
> 72 Hours (261)	17	2	13	229	57% (37-75)	99% (97-100)	89% (67-99)	95% (91-97)
Influenza B								
All (605)	**11**	**22**	**6**	**566**	**65% (38-86)**	**96% (94-98)**	**33% (18-52)**	**99% (98-100)**
≤ 72 Hours (344)	7	16	4	317	64% (31-89)	95% (92-97)	30% (13-53)	99% (97-100)
> 72 Hours (261)	4	6	2	249	67% (22-96)	98% (95-99)	40% (12-74)	99% (97-100)

There were no associations between illness severity and EHFT sensitivity (Multimedia Appendix 3); neither the number of symptoms nor disruption to daily activities were significantly associated with EHFT sensitivity, nor was there a meaningful association between either of these measures and mean influenza Crt. However, measures of illness severity were correlated with each other; individuals who reported more disruption to daily activities also reported a greater number of symptoms (r = 0.54, $P < .001$), and PCR positive individuals reported significantly higher scores for both these measures compared to PCR negative individuals ($P < .001$) (Multimedia Appendix 3).

Of the 25 FP results, the majority (22) occurred when the EHFT indicated the presence of influenza B, and all occurred within 96 hours of symptom onset (Multimedia Appendix 3). Other respiratory pathogens, namely RSV, hMPV, *human coronavirus*, and *Streptococcus pneumoniae* were detected in 7 of the 22 influenza B FP samples.

Influenza relative threshold cycles (Crt)

The average influenza Crt value for all influenza PCR positive samples was 18.82 and was significantly lower for samples collected ≤72 hours after symptom onset (17.9), than those >72 hours (20.2) ($P = .016$) (Table 4). The mean influenza Crt (16.8) for the TP individuals was significantly lower than the mean Crt value (22.0) for FN individuals ($P < .001$). Mean influenza Crt values were also significantly lower for influenza B (16.7) compared to influenza A (19.3) ($P = .037$). Multiple regression estimated the average change in Crt increases 1.34 (95% CI 0.39-2.29) Crt for each additional day after symptom onset ($P = .006$), as well as significantly lower mean Crt values with

greater number of symptoms (Multimedia Appendix 5).

Table 4: Influenza relative threshold cycles (Crt) of various subgroups

Group (N = Influenza positive participants)	Influenza Crt Raw Mean (SD)	*P* value
Influenza Subtype		
Influenza A (70)	**19.34** (4.3)	.037
Influenza B (17)	**16.71** (4.4)	
72-hour testing cutoff		
Symptom duration ≤ 72 hours (51)	**17.9** (4.4	.016
Symptom duration > 72 hours (36)	**20.14** (4.2)	
Age*		
5-17 years (11)	**16.6** (4.5)	.1
18+ years (73)	**19.1** (4.4)	
True Positives and False Negatives		
True Positives (53)	**16.8** (4.0)	< .001
False Negatives (34)	**22.0** (3.0)	

*3 flu positive participants missing age

User experiences with study procedures

Overall, participants stated they were ‘somewhat confident’ (38.5%) or ‘very confident’ (60.1%) that they completed the reference swab correctly and experienced only mild discomfort (76.8%) (Multimedia Appendix 5). This was similar for the EHFT, for which participants stated they were ‘somewhat confident’ (33.5%) or ‘very confident’ (66.1%) and experienced only mild discomfort (76.1%). Only two participants reported errors with the Bluetooth component of the EHFT device and were sent new devices. No other issues with the device were reported to the study team.

RNase P Crt from the reference sample ranged from 10.89 to 33.34 cycles (median = 21.1) and did not vary significantly between PCR positive and PCR negative samples ($P = 0.05$). We compared the RNase P average Crt values between TP, FP, FN, and TN subgroups; a Kruskal-Wallis test found a significant global *P* value ($P = 0.02$); however, Dunn's multiple comparison's test did not find significant differences between individual medians (Multimedia Appendix 5). RNase P Crt values did not vary between age groups ($P = 0.22$).

One or more errors were noted in 46 out of 587 (8%) returned kits, indicating that these participants did not correctly follow one or more of the provided instructions. Of these, 23 were returned with a packaging error (either missing the outer box or specimen bag sealed incorrectly), and 27 were returned with incorrect labeling on the VTM tube (Multimedia Appendix 5).

Discussion

Main Findings

This study demonstrated the feasibility of implementing an unsupervised at-home diagnostic test. The vast majority of participants were able to complete the multiple procedures required to evaluate a home influenza test without direct supervision, including surveys, two mid-turbinate swabs, app-guided directions to complete an influenza RDT, and returning reference samples by mail to a central laboratory. The influenza positivity within our study sample was 14.4% with 80% of those influenza A, which is consistent with both the prevalence and relative proportion of influenza strains reported in the local area during the study period [31]. The EHFT had moderate sensitivity (61%) and high specificity (95%) compared to laboratory PCR on self-collected swabs. Specificity was slightly higher for influenza A than influenza B (99% compared to 96%), while sensitivity was slightly higher for influenza B than influenza A (65% compared to 60%), although confidence intervals were wide and overlapping. The small proportion of influenza B PCR-positive participants and the high rate of influenza B FP resulted in a much lower PPV for influenza B when analyzed independently from influenza A (33% compared to 68%).

TP EHFT results had significantly lower influenza Crt values (corresponding to higher viral load) than false negative EHFT results, suggesting that lower viral load may have impacted sensitivity of EHFT. To further investigate this relationship, we assessed EHFT accuracy across two additional variables known to impact viral load, namely symptom onset and illness severity [24–26,32,33]. EHFT sensitivity was related to symptom onset, with a moderate improvement in sensitivity (6%) when the test was conducted within 72 hours of symptom onset. Furthermore, we noted a linear relationship between symptom onset and Crt value, where each additional day between symptom onset and testing corresponded to on average 1.3 additional cycles (i.e., more than a 2-fold decrease) in the estimated quantity of virus. In contrast, illness severity did not appear to influence EHFT sensitivity with no relationship observed between test accuracy and number of symptoms, nor with greater impact on daily activities. Nevertheless, these two measures of illness severity were correlated with each other, which is consistent with the expectation that individuals who report more symptoms face greater disruption to their daily activities. Notably, both measures of influenza severity were significantly higher in PCR positive individuals than in PCR negative individuals, despite not predicting viral load in this study.

We did not find any evidence that quality of self-sampling impacted EHFT accuracy, nor that particular demographic groups were more capable of collecting self-swabs than others. If sample quality impacted EHFT sensitivity or the quality of the reference swab, we would expect to see significantly higher Crt values for the endogenous control gene, RNase P, in FN and TN groups. However, we found no significant differences in comparisons between RNase P Crt values across any of the test subgroups (TP, FP, FN, TN), nor between age groups.

Comparison to prior studies

The accuracy of the EHFT we report is comparable to studies of other influenza RDTs. A 2017 meta-analysis of 134 studies of influenza RDTs showed pooled estimates of sensitivity (61%, 95%CI 53.3-68.3) and specificity (98.9%, 95% CI 98.4-99.3) [14] that are comparable to those we report. These results are consistent with two other meta-analyses [10,14]. The similarity in test accuracy is even more notable considering that published studies on influenza RDT accuracy were conducted in health care settings, with sampling and RDTs performed by health care workers/researchers rather than patients themselves.

Time from symptom onset to testing, or symptom onset, has a critical impact on viral load and influenza RDT accuracy [13,34]. One of the disadvantages of mailed testing kits is delays in testing following symptom onset due to the time needed to distribute swabbing materials [35,36]. Elliot *et al.* reported an average of 4-days between symptom onset and self-swabbing, compared to two days for clinician-collected samples. Similarly, we found that influenza Crt decreased with a longer symptom onset [35]. Studies that have lower mean times from onset to testing tend to report higher

sensitivities [13]. In contrast, one study found that testing too early can lead to increased false-negatives, primarily if the RDT is used within 12 hours of symptom onset [15], suggesting that there might be a "sweet spot" for RDT testing for influenza that must be balanced with other factors that impact test sensitivity. Additionally, we did not find a relationship between viral shedding and illness severity [24–26,32,33]. This may be because the measures of illness severity we used lack validity in our setting or population, and/or that the range of illness severities in our population was too narrow. A more robust understanding of viral load dynamics, especially in less severely ill populations, will help delineate the conditions in which an at-home RDT for influenza is most appropriate.

Quality of self-collected swabs did not appear to impact EHFT accuracy. Participants reported high confidence in completing both the EHFT swab and the reference test swab. Moreover, reference swabs had RNase P Crt values (when corrected to an equivalent CT value) that tended to be high, but within the range of those reported in other studies of both clinician-collected and self-collected mid-turbinate swabs [28,37]. EHFT swab instructions asked participants to swab both nostrils. One possible explanation for low RNase P Crt values on the reference swab, which was completed after the EHFT swab, is that there was less human cellular debris available for collection. This may also have impacted influenza Crt values. While there was variability in RNase P Crt between individuals, variability was not observed between TP, FN, FP, and TN groups, suggesting that sample collection did not impact EHFT accuracy.

Our findings of inferior accuracy of the EHFT for influenza B (including false-positive results) is consistent with other literature [38–40], and may have been due to several factors. The prevalence of influenza B in the study catchment area was low (3-4%) during the study period [31]; low disease prevalence is known to impact predictive values [41,42]. Other studies of influenza RDTs have also noted higher rates of false positives for influenza B than influenza A [38–40] suggesting non-specific reactivity with antibodies used for influenza B detection.

Strengths and Limitations

This is one of the first studies to report the accuracy of an influenza RDT used by unsupervised participants, and the first to do so for an RDT designed specifically for home use. Our study design included remote online recruitment, shipping of Flu Kits complete with RDT and reference sample collection materials, and completion of all stages of the study by the user, without direct supervision from study staff. The high response and completion rate of study procedures (75%) was matched by high self-reported confidence for both the EHFT and the reference sample procedures. There was a 20% drop-off of participants who were sent a kit but didn't participate; it is unclear if this introduced additional bias but is consistent with other mail-based testing studies [43]. While occasional errors in required shipping procedures occurred, the vast majority of samples were returned to the research laboratory in appropriate condition. Participants likely had milder symptoms than those attending clinical settings and, while this may have impacted RDT sensitivity with lower viral shedding, they represent the population in which this RDT would be used. Our findings support this type of study design for assessment of self-tests for influenza and other respiratory viruses such as SARS-CoV-2.

This study had several limitations. First, participants were more highly educated, English-speakers with private insurance and access and ability to use a blue-tooth mobile-app device. Second, many individuals (43.1%) did not conduct the EHFT within the 72 hours of illness onset, likely due to time elapsed from recruitment, shipment of kits, and participants' availability to complete the EHFT on receipt. Future studies of at-home tests should explore solutions to identify symptomatic individuals earlier in their illness and expediently provide tests. For example, cohort studies have utilized regular, self-reported symptoms surveys via SMS or email to identify influenza early and prompt testing [44,45] or through pre-positioning of Flu Kits or other at-home testing devices. Fourth, while participants reported confidence in self-collection of swabs, confirmed by markers of human DNA in these samples, there remains some uncertainty on the validity of this type of reference sample. Lastly, we recruited less than a third of the desired sample size (and only a small number of influenza B infections) due to delays in study initiation and premature closure as a result of the COVID-19

pandemic in the local area. A larger sample size would have provided tighter confidence intervals around estimates of test accuracy.

Implications for clinicians, researchers, and policy makers

RDTs designed for home use have the potential to be purchased over the counter or prescribed by health care providers, and coupled if necessary with in-person or telemedicine consultations to guide care [46]. The accuracy of the influenza EHFT reported here is similar to that of many RDTs used in clinical settings, which supports its use in similar populations, provided suitable precautions are in place, in particular to mitigate the risk of false-negative results. These could include using clinical prediction rules to assist potential RDT users in quantifying their pre-test probability of influenza. Our findings support the use of the EHFT among individuals within 72 hours of symptom onset and suggest the need for further research to understand other indicators of viral load that could be used to select individuals for whom this type of RDT should or should not be recommended.

For researchers, our study design provides a model for comparative accuracy studies of RDTs for influenza and other respiratory pathogens, including SARS-CoV-2, in home settings. We recommend that future study designs should prioritize minimizing time from symptom onset through study enrollment to conducting the index test, particularly for infections such as influenza where viral shedding declines rapidly after symptom onset. Strategies could include prepositioning test kits and encouraging early completion of the RDT with onset of illness. Given the potential important relationship between influenza severity and viral load (and hence self-test sensitivity), we also encourage use of more accurate ways to measure illness severity from self-reported surveys. Finally, rather than blinding participants to RDT results, revealing self-test results would facilitate recruitment and allow exploration of impacts of positive and negative self-test results on participants' health-seeking and other behaviors.

Conclusion

The EHFT showed comparable accuracy to influenza RDTs used in clinical settings. The EHFT was most sensitive when used within 72 hours of symptom onset. Home tests have the potential to expand access to testing for infectious diseases, with potential benefits for individuals and the health care system.

Acknowledgments:
Kit Fabrication: We'd like to acknowledge Rosemichelle Marzan, Grace Kim, Ashley Song, Kendall Escene, and Angel Wong for their critical help constructing study kits.
Shipping Operations: We'd like to acknowledge Lincoln C. Pothan and her logistics team for orchestrating the extremely expedient shipping and distribution of test kits to participants.
Marketing: We'd like to acknowledge Jaclyn Ruckle, Jen Mooney, and the Formative team, for designing persuasive advertisements to assist with study recruitment.

Conflicts of Interest: H.Y.C. has received research support from GlaxoSmithKline, Novavax, and Sanofi Pasteur. B.L. is co-founder of Anavasi Diagnostics, Inc. that is developing point-of-care diagnostics. M.J.T. has received reimbursement as medical advisor for Click Diagnostics (now Visby Medical), Inflammatrix, and Roche Molecular Diagnostics which are all companies that are involved in point of care tests for infectious disease pathogens.

References:

1. CDC. Preliminary In-Season 2019-2020 Flu Burden Estimates. *Centers for Disease Control and Prevention*, https://www.cdc.gov/flu/about/burden/preliminary-in-season-estimates.htm (2020, accessed 12 May 2020).
2. Putri WCWS, Muscatello DJ, Stockwell MS, et al. Economic burden of seasonal influenza in the United States. *Vaccine* 2018; 36: 3960–3966.
3. Clinical Laboratory Improvement Amendments (CLIA) | CMS, https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA (accessed 12 May 2020).
4. Basile K, Kok J, Dwyer DE. Point-of-care diagnostics for respiratory viral infections. *Expert Rev Mol Diagn* 2018; 18: 75–83.
5. Egilmezer E, Walker GJ, Bakthavathsalam P, et al. Systematic review of the impact of point-of-care testing for influenza on the outcomes of patients with acute respiratory tract infection. *Rev Med Virol* 2018; 28: e1995.
6. Qualls N, Levitt A, Kanade N, et al. Community Mitigation Guidelines to Prevent Pandemic Influenza - United States, 2017. *MMWR Recomm Rep* 2017; 66: 1–34.
7. Fong MW, Gao H, Wong JY, et al. Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings-Social Distancing Measures. *Emerg Infect Dis* 2020; 26: 976–984.
8. Seaman CP, Tran LTT, Cowling BJ, et al. Self-collected compared with professional-collected swabbing in the diagnosis of influenza in symptomatic individuals: A meta-analysis and assessment of validity. *J Clin Virol* 2019; 118: 28–35.
9. Chartrand C, Leeflang MMG, Minion J, et al. Accuracy of rapid influenza diagnostic tests: a meta-analysis. *Ann Intern Med* 2012; 156: 500–511.
10. Merckx J, Wali R, Schiller I, et al. Diagnostic Accuracy of Novel and Traditional Rapid Tests for Influenza Infection Compared With Reverse Transcriptase Polymerase Chain Reaction: A Systematic Review and Meta-analysis. *Ann Intern Med* 2017; 167: 394–409.
11. Rumbold B, Wenham C, Wilson J. Self-tests for influenza: an empirical ethics investigation. *BMC Med Ethics* 2017; 18: 33.
12. Green DA, StGeorge K. Rapid Antigen Tests for Influenza: Rationale and Significance of the FDA Reclassification. *J Clin Microbiol*; 56. Epub ahead of print October 2018. DOI: 10.1128/JCM.00711-18.
13. Chan MCW, Lee N, Ngai KLK, et al. Clinical and virologic factors associated with reduced sensitivity of rapid influenza diagnostic tests in hospitalized elderly patients and young children. *J Clin Microbiol* 2014; 52: 497–501.
14. Bruning AHL, Leeflang MMG, Vos JMBW, et al. Rapid Tests for Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review and Meta-analysis. *Clin Infect Dis* 2017; 65: 1026–1032.
15. Tanei M, Yokokawa H, Murai K, et al. Factors influencing the diagnostic accuracy of the rapid influenza antigen detection test (RIADT): a cross-sectional study. *BMJ Open* 2014; 4: e003885.
16. Thompson M, Zigman Suchsland ML, Lyon V, et al. 1775. A Community-wide Study to Evaluate the Accuracy of Self-testing for Influenza: Works in Progress. In: *Open Forum Infectious Diseases*. Oxford University Press US, 2019, pp. S654–S654.
17. Chu HY, Boeckh M, Englund JA, et al. The Seattle Flu Study: a multiarm community-based prospective study protocol for assessing influenza prevalence, transmission and genomic epidemiology. *BMJ Open* 2020; 10: e037295.
18. Cohen JF, Korevaar DA, Altman DG, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *BMJ Open* 2016; 6: e012799.
19. Powers JH, Guerrero ML, Leidy NK, et al. Development of the Flu-PRO: a patient-reported outcome (PRO) instrument to evaluate symptoms of influenza. *BMC Infect Dis* 2016; 16: 1.

20. Ebell MH, Afonso AM, Gonzales R, et al. Development and validation of a clinical decision rule for the diagnosis of influenza. *J Am Board Fam Med* 2012; 25: 55–62.
21. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; 42: 377–381.
22. World Health Organization. *Guidance on regulations for the transport of infectious substances 2019--2020*. World Health Organization, https://apps.who.int/iris/bitstream/handle/10665/325884/WHO-WHE-CPI-2019.20-eng.pdf (1 January 2019).
23. World Health Organization. *Guidance on regulations for the transport of infection substances*, https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2.pdf (1 January 2007).
24. Lee N, Chan PKS, Hui DSC, et al. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. *J Infect Dis* 2009; 200: 492–500.
25. Rodrigues Guimarães Alves V, Perosa AH, de Souza Luna LK, et al. Influenza A(H1N1)pdm09 infection and viral load analysis in patients with different clinical presentations. *Mem Inst Oswaldo Cruz* 2020; 115: e200009.
26. Lau LLH, Cowling BJ, Fang VJ, et al. Viral shedding and clinical illness in naturally acquired influenza virus infections. *J Infect Dis* 2010; 201: 1509–1516.
27. Note A. Crt, a relative threshold method for qPCR data analysis on the Quant Studio 12K Flex system with Open Array technology, https://assets.thermofisher.com/TFS-Assets/LSG/brochures/CO28730-Crt-Tech-note_FLR.pdf.
28. Van Wesenbeeck L, Meeuws H, D'Haese D, et al. Sampling variability between two mid-turbinate swabs of the same patient has implications for influenza viral load monitoring. *Virol J* 2014; 11: 233.
29. World Health Organization (WHO). CDC protocol of realtime RTPCR for swine influenza A (H1N1). *CDC protocol of realtime*, https://pesquisa.bvsalud.org/portal/resource/pt/des-17766 (2009).
30. [R Core Team]. R: A language and environment for statistical computing, http://finzi.psych.upenn.edu/R/library/dplR/doc/intro-dplR.pdf (2013).
31. Public Health-Seattle & King County. Influenza. *Influenza - King County*, https://www.kingcounty.gov/depts/health/communicable-diseases/disease-control/influenza.aspx (2020, accessed 15 September 2020).
32. de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006; 12: 1203–1207.
33. Bagga B, Woods CW, Veldman TH, et al. Comparing influenza and RSV viral and disease dynamics in experimentally infected adults predicts clinical effectiveness of RSV antivirals. *Antivir Ther* 2013; 18: 785–791.
34. Cheng CKY, Cowling BJ, Chan KH, et al. Factors affecting QuickVue Influenza A + B rapid test performance in the community setting. *Diagn Microbiol Infect Dis* 2009; 65: 35–41.
35. Elliot AJ, Bermingham A, Charlett A, et al. Self-sampling for community respiratory illness: a new tool for national virological surveillance. *Euro Surveill* 2015; 20: 21058.
36. Zigman Suchsland, ML, Rahmatullah, I, Lutz, B, Lyon, L, Huang, S, Kline, E, Graham, C, Cooper, S, Su, P, Smedinghoff, S, Chu, HY, Sewalk, K, Brownstein, JS, Thompson, MJ, on behalf of Seattle Flu Study investigators. Evaluating an app-guided self-test for influenza: lessons learned for improving the feasibility of study designs to evaluate self-tests for respiratory viruses. *[Manuscript submitted for publication] BMC Infectious Disease*.
37. Thompson MG, Ferber JR, Odouli R, et al. Results of a pilot study using self-collected mid-

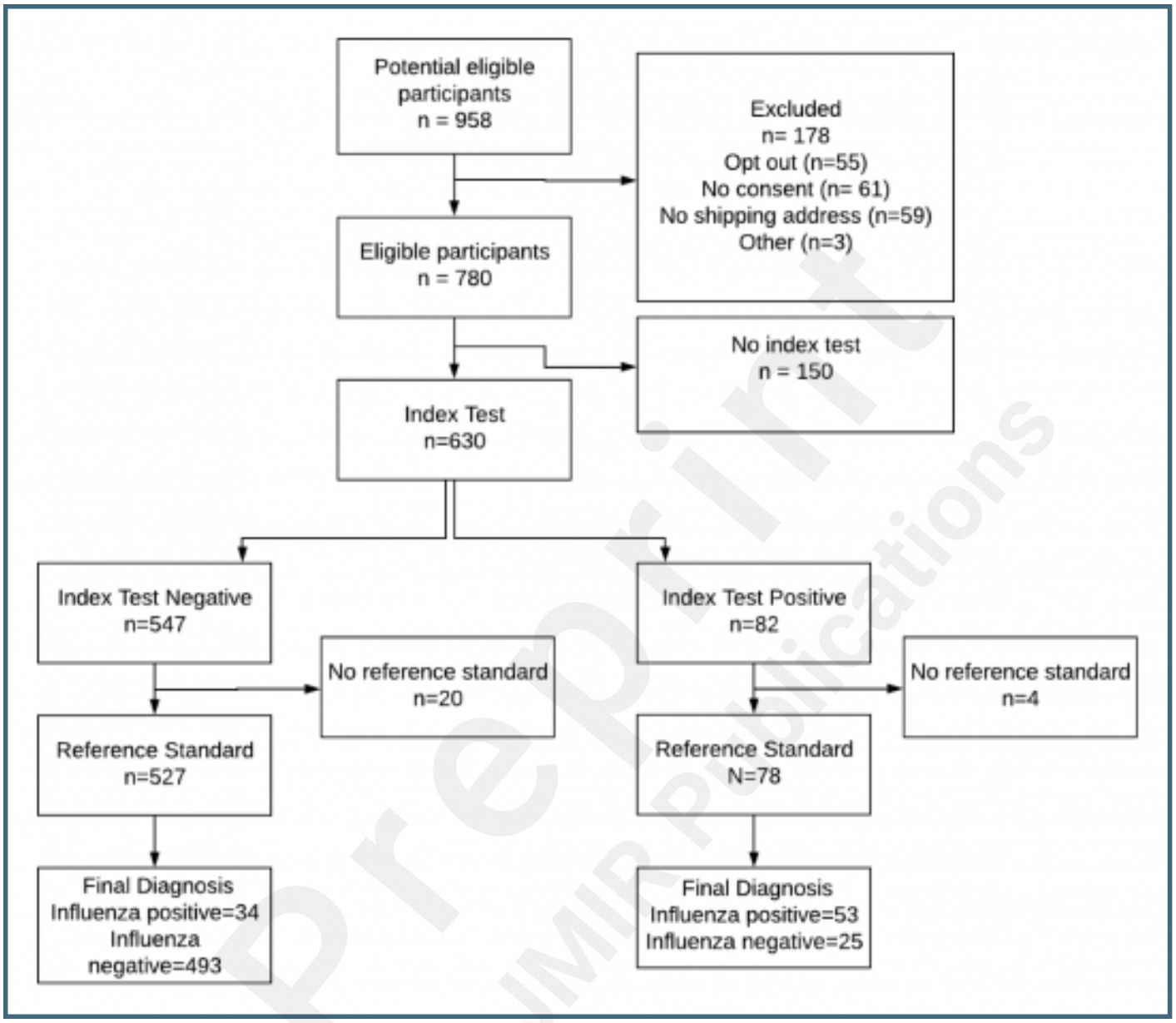
turbinate nasal swabs for detection of influenza virus infection among pregnant women. *Influenza Other Respi Viruses* 2015; 9: 155–160.
38. Iovine NM, Ison T, Payton T, et al. High percentage of false-positive results for influenza B obtained with a rapid influenza point-of-care test. *Clin Infect Dis* 2014; 59: 604–605.
39. Nivin B, Zancocchio T. Cluster of false-positive influenza B virus rapid antigen test results in a New York City hospital. *J Clin Microbiol* 2012; 50: 3141.
40. Otto MP, Toyer A-L, Poggi C, et al. Influenza B false-positive results by rapid molecular tests Alere™ i Influenza A&B 2 in France. *Diagn Microbiol Infect Dis* 2019; 94: 342–343.
41. Leeflang MMG, Rutjes AWS, Reitsma JB, et al. Variation of a test's sensitivity and specificity with disease prevalence. *CMAJ* 2013; 185: E537–44.
42. Grijalva CG, Poehling KA, Edwards KM, et al. Accuracy and interpretation of rapid influenza tests in children. *Pediatrics* 2007; 119: e6–11.
43. Sanner K, Wikström I, Strand A, et al. Self-sampling of the vaginal fluid at home combined with high-risk HPV testing. *Br J Cancer* 2009; 101: 871–874.
44. Marinsek N, Shapiro A, Clay I, et al. Measuring COVID-19 and Influenza in the Real World via Person-Generated Health Data. *Infectious Diseases (except HIV/AIDS)*. Epub ahead of print 30 May 2020. DOI: 10.1101/2020.05.28.20115964.
45. Smolinski MS, Crawley AW, Baltrusaitis K, et al. Flu Near You: Crowdsourced Symptom Reporting Spanning 2 Influenza Seasons. *Am J Public Health* 2015; 105: 2124–2130.
46. Lyon V. Feasibility and Acceptance of Mobile Point-of-Care Self Testing: A Case Study on the Flu@home Mobile App.

Abbreviations:
acute respiratory illness (ARI);
cycle threshold (CT);
relative cycle threshold (Crt);
Ellume home flu test (EHFT);
false negative (FN);
false positive (FP);
point-of-care (POC);
rapid diagnostic test (RDT);
Research Electronic Data Capture (REDcap);
Ribonuclease P (RNase P);
quantitative reverse-transcription polymerase chain reaction (RT-qPCR or PCR)
Seattle Flu Study (SFS);
true positive (TP);
true negative (TN);
viral transport medium (VTM)

Supplementary Files

Figures

Recruitment flow for the Home Flu Test participants.



Multimedia Appendixes

Additional details on participant data collection.
URL: http://asset.jmir.pub/assets/a11c34106213718069ac4c1d11650d94.docx

Additional participant demographics.
URL: http://asset.jmir.pub/assets/a54e4ae70f5134e7fcf8732cf25877bd.docx

Additional details on RDT accuracy.
URL: http://asset.jmir.pub/assets/6870e0eeda4645b44edc28d48375ba4d.docx

Regression adjusted mean Crt.
URL: http://asset.jmir.pub/assets/69e2ac98c27c5d1d39139da0ff33b543.docx

User experience with study procedures.
URL: http://asset.jmir.pub/assets/94743ccd1f688188e92c951299297446.docx