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Facemasks, Hand Hygiene, and Influenza among Young Adults: A Randomized Intervention Trial

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Abstract

Limited vaccine availability and the potential for resistance to antiviral medications have led to calls for establishing the efficacy of non-pharmaceutical measures for mitigating pandemic influenza. Our objective was to examine if the use of face masks and hand hygiene reduced rates of influenza-like illness (ILI) and laboratory-confirmed influenza in the natural setting. A cluster-randomized intervention trial was designed involving 1,178 young adults living in 37 residence houses in 5 university residence halls during the 2007–2008 influenza season. Participants were assigned to face mask and hand hygiene, face mask only, or control group during the study. Discrete-time survival models using generalized estimating equations to estimate intervention effects on ILI and confirmed influenza A/B infection over a 6-week study period were examined. A significant reduction in the rate of ILI was observed in weeks 3 through 6 of the study, with a maximum reduction of 75% during the final study week (rate ratio [RR] = 0.25, [95% CI, 0.07 to 0.87]). Both intervention groups compared to the control showed cumulative reductions in rates of influenza over the study period, although results did not reach statistical significance. Generalizability limited to similar settings and age groups. Face masks and hand hygiene combined may reduce the rate of ILI and confirmed influenza in community settings. These non-pharmaceutical measures should be recommended in crowded settings at the start of an influenza pandemic.

Trail Registration: Clinicaltrials.gov NCT00490633

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Introduction

As part of planning for pandemic influenza, serious attention has been given to non-pharmaceutical interventions (NPIs) for prevention. This is based in part on the realization that vaccines and antiviral medications may be in short supply or unavailable at the start of a pandemic. A number of studies have recently been conducted to strengthen the scientific basis for recommendations on the use of specific influenza interventions [1]. These studies have primarily been performed during seasonal influenza outbreaks, but have recently been validated during the swine origin pandemic of 2009, in which the need for NPIs was reaffirmed. NPIs implemented at the beginning of the 2009 pandemic included home quarantine, isolation of the ill, social distancing, and personal protection measures (e.g. face masks and hand hygiene).

We began to examine the impact of NPIs on the incidence of seasonal influenza in 2006–2007, a year of modest influenza activity [2]. Our work demonstrated that the use of face masks and hand hygiene, combined, conferred protection from primary influenza-like illness (ILI) among young adults living in university residence halls [2]. We continued our research into the 2007–2008 influenza season with improvements in our study design aimed at a more efficient examination of intervention effects on rates of ILI and laboratory-confirmed influenza. In contrast to our earlier study, the 2007–2008 season was characterized by higher levels of influenza activity. In this paper, we present findings from the 2007–2008 season of our cluster-randomized intervention trial of face masks and hand hygiene for preventing ILI and laboratory-confirmed influenza.

Methods

The protocol and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1.

Ethics statement

The study was approved by the Institutional Review Board at the University of Michigan, HUM00008566. Informed consent was obtained by all participants through electronic signature of the online form, as approved by the Institutional Review Board.

Study design

A cluster-randomized intervention trial (Mflu) was conducted at the University of Michigan (trial registration: Intervention Study of

Face Mask and Hand Sanitizer to Reduce Influenza Transmission (M-FLU), Identifier: NCT00490633, trial link: http://www.clinicaltrials.gov/ct2/show/NCT00490633). Findings from the first year of Mflu for the 2006–2007 flu season have been published [2]. The 2007–2008 trial described here followed 1,178 young adults living within university residence halls during the influenza season and included a significantly larger number of clusters for randomization. Thirty-seven residence houses located in five residence halls were randomly assigned to either an intervention or a control group. Students living in these residence halls were eligible for the study if they were at least 18 years of age, willing to wear a face mask, use alcohol-based hand sanitizer, provide a throat swab specimen when sick, and complete one baseline and six weekly on-line surveys. Students reporting an allergy to alcohol-based hand sanitizer were excluded. Based on data from year one of the Mflu study [2] and assuming an 8% observable ILI attack rate in the control group, we had 87% power to detect a reduction of 25% (i.e. a rate ratio [RR] = 0.75) or greater in illness rates between intervention and control groups at an α-level 0.05, using the methods of Hayes et al for cluster randomized trials [3]. The CONSORT checklist is presented in Checklist S1.

Randomization and intervention

Randomization at the residence house level was performed using Proc Plan (SAS v. 9.1 Cary, NC) by study staff (see Text S1 section I for further details on randomization). A residence house is defined as a shared "hall way", "wing", or "floor (s)" containing several dorm rooms that share common areas or bathrooms. For participating residence halls, the average number of residence houses in each was 7.4 (range: 4 to 9). All residence houses in each of the residence halls were randomized prior to the intervention implementation. Recruitment by study staff began in November 2007 and continued through February 2008. The intervention period began during the week of January 28, 2008 following laboratory-confirmation of influenza on campus through ongoing surveillance at the University Health Services. Intervention materials and a required educational video on proper hand hygiene and use of standard medical procedure face masks were provided to study participants on January 24th. There was also a one-week spring break during the study when a majority of students left campus (February 23rd–March 2nd) and therefore illness symptoms that may have occurred during this period were not assessed. Excluding the break, interventions were implemented for 6 weeks (i.e. 42 days) and ended on March 14th.

The intervention groups included mask and hand hygiene or mask alone. Participants in the face mask and hand hygiene and the face mask only groups received weekly packets of mask supplies in their student mailboxes. Each packet included seven standard medical procedure masks with ear loops (TECNOL^{TM} procedure masks, Kimberly-Clark, Roswell GA) and plastic bags for storage during interruptions in mask use (e.g., while eating, sleeping, etc.) and for daily disposal. Participants were asked to wear their masks for at least six hours per day while in their residence hall. Students were encouraged but not obligated to wear their face masks outside of their residence hall. In addition to masks, all participants in the face mask and hand hygiene intervention received hand sanitizer (2 oz squeeze bottle, 8 oz pump bottle with 62% ethyl alcohol in a gel base). The control group did not receive an intervention. Additional information on supply distribution is presented in (Text S1 section I).

Weekly surveys

Participants were asked to provide self-reported data at baseline on demographic information, hand hygiene practices, health behaviors, smoking habits, vaccination, and perceived stress [4]. Participants were also asked to complete on-line weekly surveys and to report the presence/absence of illness symptoms. Weekly surveys included questions on ILI symptoms, intervention compliance (e.g. total mask hours per day and frequency of alcohol-based hand sanitizer use), and health and hand hygiene practices. Detailed descriptions of additional behavioral and compliance measures are presented online in Text S1 (see sections II and III).

ILI symptoms and laboratory testing

All study participants were given materials describing the ILI case definition (presence of cough and at least one or more of fever/feverishness, chills, or body aches) and contact information of clinical research staff for illness assessment. Clinical research staff recorded the date of illness onset, body temperature, use of anti-pyretics, and reported symptoms. Throat swab specimens were tested for influenza A or B using real-time polymerase chain reaction (Rt-PCR). Positive samples were identified using PCR samples tested using the TaqMan System (Applied Biosystem, Foster City, CA, USA). Primers and probes were developed by the CDC Influenza Branch to detect influenza types A and B as previously noted [5]. Information on laboratory procedures are included online in Text S1 section IV.

Statistical analysis

The objective of this study was to assess whether the application of masks or masks and hand hygiene together among a generally health student population reduces influenza and ILI compared to a control group not receiving these interventions. We hypothesized that there would be a significant reduction in influenza and ILI in both the mask and hand hygiene and mask alone groups compared to the control group. This was a single blind study where the PI's and statisticians were blinded to intervention status during analyses. Imbalances in baseline study characteristics were examined between intervention and control groups using cluster-adjusted chi-squared tests and cluster-adjusted ANOVA [6]. Intracluster correlation coefficients (ICCs) and corresponding *P* values were calculated in R software using the Donner method [7,8].

Compliance. Compliance measures were log transformed to account for skewness. Values of 0 were given a value of 1 prior to log transformation. Differences in compliance between intervention and control groups were examined using multi-level mixed models in SAS Proc Mixed (SAS V.9.1, Cary, NC). Level-1 accounted for changes between individuals in compliance over the course of the study period. Level-2 allowed for changes in compliance within individuals across the study period. Random intercepts were used to account for clustering by residence house. The type III F-test and corresponding *P* value were computed for an overall comparison in compliance between the intervention and control groups. Weekly comparisons between groups were also evaluated. *P* values were set to $\leq$0.025 for statistical significance to account for multiple comparisons in week-by-week analyses.

Intervention. The main predictor variable was an indicator for whether the individual was in an intervention group (mask and hand hygiene or mask alone) or control group (as the referent). The main outcome variables measured at the individual level were time to first ILI and time to PCR-confirmed influenza A/B during the study period. In total, 1,178 of 1,188 recruited students living in participating residence halls were eligible for study inclusion (see Figure 1). Of the 1,178 participants, 1,111 were available for statistical analyses (see Figure 1). For incident ILI, an ILI-free study population at baseline was examined (N = 938/1,111). For

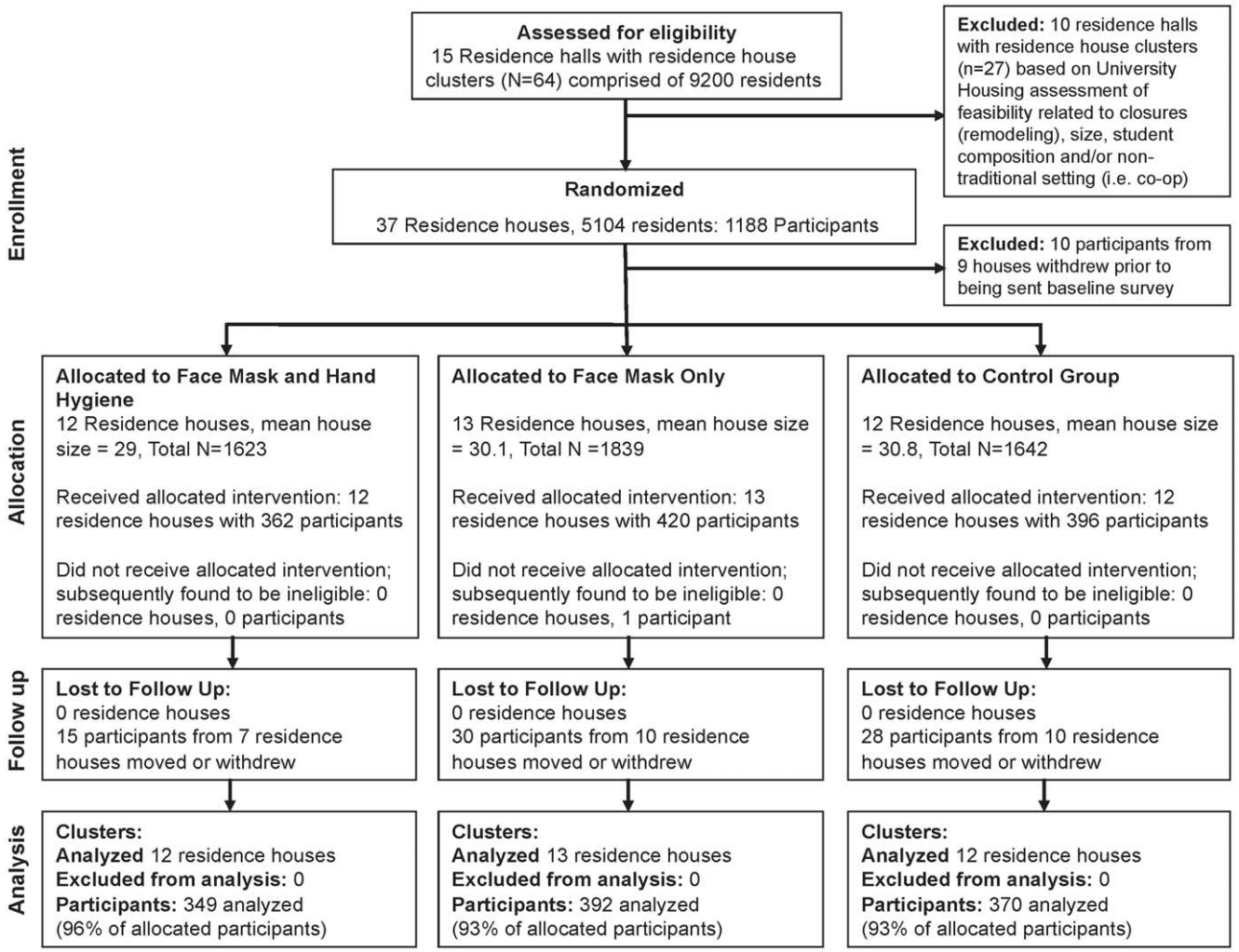

Figure 1. Flow chart of participants throughout the study period. This figure shows the enrollment, allocation, follow-up, and analysis numbers for the study.
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influenza, all participants (N = 1,111) were examined since there were no laboratory confirmed cases of influenza at baseline.

An intention-to-treat analysis was performed [9]. Log-survival and log-log survival plots showed that the proportional hazards assumption was not met. Therefore, discrete-time survival analysis was performed to examine intervention effects on time to first ILI or influenza infection [10]. Proc Genmod in SAS (SAS V.9.1, Cary, NC) was used to estimate rate ratios (RRs) and 95% confidence intervals (CIs) for each study week and cumulatively over the study period for ILI. Only cumulative RRs were examined for influenza since the number of cases was too small to generate weekly estimates. Generalized estimating equations were used to control for clustering [10,11]. Variables measured at baseline were added to the final model if the magnitude of effect was more than a 10% increase or decrease from the null value (RR = 1.00) in univariate analyses. RRs were considered statistically significant at $P<0.05$.

Results

Demographics

A total of 1,111 eligible participants (94% retention) were available for analysis with 349 in face mask and hand hygiene, 392 in mask only, and 370 in the control group (see Figure 1). Baseline characteristics of participants are shown in Table S1. The mean age was 18.95 years (SD, 0.9). There were no statistically significant differences between study groups among any of the covariates examined (see Table S1).

Compliance

Compliance analyses demonstrated that subjects in the face mask and hand hygiene group wore their mask, on average, 5.08 hours per day (SD, 2.23) compared to subjects in the mask only group (5.04 hours per day [SD, 2.20]) (see Figure 2). No significant difference in mask use between the two interventions was observed throughout the study (see Figure 2).

Alcohol-based hand sanitizer use (study provided or personally owned) was examined among subjects in each of the three study groups. The face mask and hand hygiene group reported an average use of hand sanitizer 4.49 times per day (SD, 4.10). The mask only group reported an average use of hand sanitizer 1.29 times per day (SD, 1.77) and the control group reported use of 1.51 times per day (SD, 2.25). The face mask and hand hygiene group used hand sanitizer significantly more often compared to subjects in either the mask only or control groups (see Table 1). No significant differences were observed between the mask only and

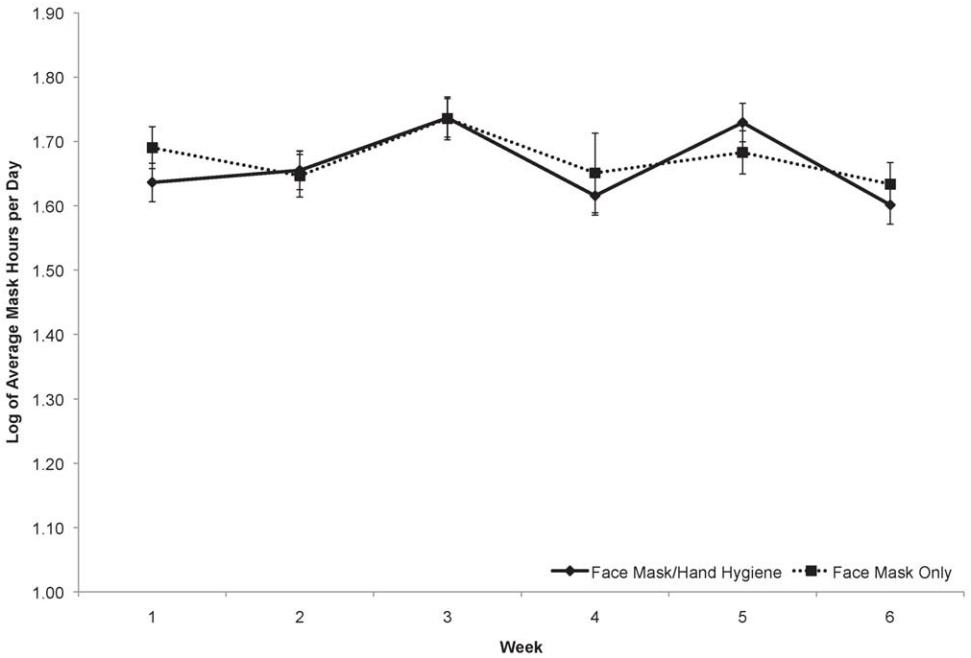

Figure 2. Reported daily average number of hours (log transformed) of facemask use by study week. This figure shows the daily average number of hours (log transformed) of facemask use by study week in both the face mask and hand hygiene group (solid line) and the face mask only group (dotted line). The type III fixed effects model for assessing differences over time using a week * group interaction term, was not statistically significant, $F(5, 2943) = 1.30$, $P = 0.26$.
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control group. Results for additional compliance measures are described in Text S1 section III and shown in tables S2, S3, S4, S5, S6, S7 and figures S1, S2, S3, S4.

ILI and laboratory-confirmed influenza

One hundred and seventy three of 1,111 participants reported ILI on their baseline survey. The remaining 938 subjects were considered ILI-free and analyzed for incident ILI during the study period. Factors associated with incident ILI included gender, race, ethnicity, smoking, physical activity, and having ever received an influenza vaccination (see Table 2). The proportion of subjects with ILI and laboratory-confirmed influenza by study group are shown in Table S5. Of the 938 ILI-free participants at baseline, 128 (14%) subsequently met the case definition of ILI throughout the study period. Of these 128 ILI cases, 34 subjects tested positive by Rt-PCR for influenza infection (27%).

At week 3 and onward, significantly reduced ILI rates were observed in the face mask and hand hygiene group compared to the control in adjusted models (see Table 3). The largest reduction was observed during week 6 with a 75% reduced ILI rate (adjusted RR = 0.25, [95% CI, 0.07 to 0.87]) among subjects in the face mask and hand hygiene group in adjusted models. Statistically significant findings were not observed for the face mask only group when compared to the control group (see Table 3).

Table 4 shows the cumulative RRs for laboratory-confirmed influenza. The face mask and hand hygiene group and the face mask only group compared to the control showed a 43% (adjusted RR = 0.57, [CI, 0.26 to 1.24]) and 8% (adjusted RR = 0.92, [CI,

Table 1. Reported average (log-transformed) daily alcohol-based hand sanitizer use per week.

Log reported average daily alcohol based hand sanitizer use per week and *P* values comparing average use in each group with face mask and hand hygiene

Intervention	Average use over all weeks[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Face Mask and Hand Hygiene	1.49	1.47	1.48	1.53	1.37	1.40	1.40
vs. Face Mask Only[b]	0.61	0.65	0.61	0.62	0.58	0.57	0.60
		(*P*<.001)	(*P*<.001)	(*P*<.001)	(*P*<.001)	(*P*<.001)	(*P*<.001)
vs. Control	0.66	0.70	0.70	0.69	0.59	0.62	0.65
		(*P*<.001)	(*P*<.001)	(*P*<.001)	(*P*<.001)	(*P*<.001)	(*P*<.001)

[a]The change in reported average log transformed daily alcohol-based hand sanitizer use over the 6 week period comparing between all three study groups (week*group interaction term) using a Type III fixed effects model resulted in an $F(10, 4431) = 1.18$ and $P = 0.30$.
[b]There were no statistically significant differences at any weeks comparing hand sanitizer use between face mask only and the control group (all $P > 0.025$).
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Table 2. Unadjusted associations between demographic characteristics and rate of influenza-like illness among subjects who were ILI free at baseline (N = 938).

Characteristic	ICC[a]	RR	95% CI	P[b]
Age at baseline	0.0019	1.06	(0.87–1.28)	0.58
Gender female vs. male	0.0014	1.61	(1.11–2.33)	0.01
Race (ref White)	0.0014			
black		0.28	(0.09–0.87)	0.03
Asian		0.90	(0.57–1.41)	0.63
Other		0.56	(0.22–1.37)	0.20
Ethnicity				
Hispanic or Latino vs. not Hispanic or Latino	0.0018	1.69	(0.88–3.24)	0.11
Sleep quality good vs. bad	0.0017	1.01	(0.65–1.56)	0.98
Stress score	0.0017	0.99	(0.97–1.01)	0.33
Smoking current vs. non	0.0011	2.39	(1.04–5.49)	0.04
Alcohol consumption (ref 0 drinks/week)	0.0013			
1 or more drinks		1.03	(0.70–1.50)	0.90
Physical activity high vs. low	0.0013	1.30	(0.90–1.89)	0.16
Flu shot ever vs. never	0.0017	1.27	(0.88–1.81)	0.20
Recent shot yes vs. no	0.0019	0.95	(0.59–1.52)	0.82
Optimal handwashing at baseline yes vs. no	0.0019	0.88	(0.58–1.34)	0.56
Hand sanitizer ownership yes vs. no	0.0017	1.00	(0.70–1.42)	0.99

[a]ICC = Intracluster correlation coefficient; RR = rate ratio; 95% CI = 95% confidence interval.
[b]Variables added to the final adjusted model if the magnitude of effect was more than a 10% increase or decrease from the null value (RR = 1.00).
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0.59 to 1.42]) reduction in the cumulative rate of influenza, respectively, throughout the study.

Discussion

We examined the efficacy of face masks and hand hygiene for reducing the incidence of ILI and laboratory-confirmed influenza in an open, non-institutionalized population of young adults. Our findings show a significant reduction in the rate of ILI among participants randomized to the face mask and hand hygiene intervention during the latter half of the study period, ranging from 48% to 75% when compared to the control group. We also observed a substantial (43%) reduction in the incidence of influenza infection in the face mask and hand hygiene group compared to the control, but this estimate was not statistically significant. There were no substantial reductions in ILI or laboratory-confirmed influenza in the face mask only group compared to the control. Our ILI findings are consistent with results from the first year of this two-year study [2] and a previous literature review on studies examining the efficacy of mask use in reducing transmission of respiratory viruses [12]. There are no other mask and hand hygiene intervention studies, to our knowledge, that have examined if wearing a mask prior to illness and jointly practicing hygiene prevents illness for the person practicing the intervention. The majority of earlier studies examined the impact of wearing a mask after a household member had been identified as an ILI or influenza case [13,14,15,16]. Our study, therefore, is an important contribution to understanding the effectiveness of these interventions for mitigating influenza outbreaks and possibly pandemic scenarios in crowded and close living environments before outbreaks ensue.

Although few data are available to evaluate the efficacy of mask use in the community setting [17], four recent randomized mask intervention trials examined the impact of mask use on secondary transmission of ILI, upper respiratory infection and/or influenza in households [13,14,15,16]. Cowling et al. [13] showed a reduction of 67% in influenza infection when masks were donned within 36 hours of the index case's symptom onset. Canini et al. [16] found no association between intervention households providing the primary case with masks compared to control households with no masks; the authors did, however, report a severely underpowered study due to early termination of the intervention. MacIntyre et al. [14] showed a borderline significant reduction in ILI among study participants using P2 masks but only 21% complied with mask use. Larson et al. [15] found no significant difference between targeted education, education with use of hand sanitizer, and education with masks and hand hygiene for overall rates of upper respiratory infection (URI); but, face masks were associated with a reduced secondary attack rate [15]. In contrast to these earlier studies, our design allowed us to follow disease-free participants at baseline who were asked to wear masks and/or conduct hygiene for the entire follow-up period, not just when they or their contacts were ill, thus limiting the potential for infection prior to mask adoption. Furthermore, our study design more accurately represents guidelines and plans that call for use of NPIs before susceptible individuals become ill [18].

In both this study and in our earlier work [2] we identified significant reductions in ILI rates several weeks into the study. Increases in compliance with hand hygiene measures may partly explain why we observed a significant reduction during the latter half of the study period over two different influenza seasons with different participants and cluster sample sizes [2]. First, a significantly higher proportion of those in the face mask and hand hygiene group reported using at least a quarter size or greater amount of hand sanitizer (equivalent to at least one full pump from an 8 oz. bottle) compared to both the face mask only

Table 3. Intervention rate ratios for influenza-like illness.

	Unadjusted Model[a]					
	Face Mask vs. Control			Face Mask/Hand hygiene vs. Control		
Week	RR[b]	95% CI[c]	*P*	RR[b]	95% CI[c]	*P*
1	0.80	(0.41–1.53)	0.49	0.99	(0.51–1.93)	0.98
2	0.86	(0.52–1.40)	0.53	0.78	(0.47–1.29)	0.33
3	0.92	(0.62–1.37)	0.68	0.61	(0.37–1.01)	0.06
4	0.99	(0.64–1.52)	0.96	0.48	(0.24–0.94)	0.03[e]
5	1.06	(0.61–1.87)	0.83	0.38	(0.15–0.94)	0.04[e]
6	1.14	(0.54–2.41)	0.72	0.30	(0.09–0.98)	0.05
Cumulative Rate Ratio[d]	1.08	(0.86–1.34)	0.52	0.78	(0.59–1.05)	0.10
	Adjusted Model[a]					
	Face Mask vs. Control			Face Mask/Hand hygiene vs. Control		
1	0.64	(0.34–1.19)	0.16	0.85	(0.44–1.64)	0.62
2	0.70	(0.44–1.14)	0.15	0.66	(0.40–1.10)	0.11
3	0.77	(0.51–1.17)	0.23	0.52	(0.30–0.88)	0.02[e]
4	0.85	(0.53–1.36)	0.49	0.40	(0.20–0.83)	0.01[e]
5	0.93	(0.51–1.71)	0.82	0.32	(0.12–0.84)	0.02[e]
6	1.02	(0.46–2.25)	0.96	0.25	(0.07–0.87)	0.03[e]
Cumulative Rate Ratio[d]	1.10	(0.88–1.38)	0.42	0.78	(0.57–1.08)	0.13

[a]Intracluster correlation coefficient: 0.0004 in unadjusted model (N = 938), −0.0005 in model adjusting for gender, race, ethnicity, smoking status, physical activity, and having ever received a vaccination for influenza (N = 828).
[b]RR, rate ratio.
[c]95% CI, 95% confidence interval.
[d]Cumulative rate ratio is the week by treatment effect which is equivalent to the hazard ratio over the study period.
[e]Significance level set at $P<0.05$.
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and control groups starting at week 4 in this study (see Text S1 section III). Hence, greater adherence with hand sanitizer use throughout the study period and a significant difference in the amount used in the latter half of this period may have contributed to the increased reductions in ILI rates observed during the latter half of the study period. Next, in both our 2006–07 and 2007–08 studies, we increased notifications regarding mask compliance to subjects in either mask intervention group and hand hygiene compliance to subjects in the face mask and hand hygiene group when they returned from spring break. Spring break occurred before the fourth week of the study and this may have led to improved compliance with hand hygiene and mask hours during the latter half of the period when compliance messaging was enhanced. Although we did not identify a significant increase in mask compliance during this time, it is possible that the participants became more comfortable with proper use of the masks as the study progressed. We were able to collect survey information over the study period on mask comfort and found that there was a slight increase in reported comfort for the mask groups beginning at week four (see Text S1 section III).

Our RRs for comparing the face mask and hand hygiene group to the control group suggest an overall reduction in the primary incidence of influenza. However, we only analyzed 34 incident cases of influenza during the study and this limited our statistical

Table 4. Intervention rate ratios for influenza infection.

Unadjusted Model[a]					
Face Mask vs. Control			Face Mask/Hand hygiene vs. Control		
cRR[b]	95% CI[c]	*P* Value	RR[b]	95% CI[c]	*P* Value
0.93	(0.60–1.42)	0.72	0.57	(0.26–1.24)	0.15
Adjusted Model[a]					
0.92	(0.59–1.42)	0.69	0.57	(0.26–1.24)	0.16

[a]Intracluster correlation coefficient: −0.0014 in unadjusted model (N = 1,111), −0.0030 in model adjusting for gender, race, ethnicity, smoking status, physical activity, and having ever received a vaccination for influenza (N = 986).
[b]cRR = Cumulative Rate Ratio is the week by treatment effect which is equivalent to the hazard ratio over the study period.
[c]95% CI, 95% confidence interval. Significance level set at $P<0.05$.
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power. Nonetheless, the RR for the layered intervention group was of even greater magnitude and in the same direction as the cumulative RR for ILI. These trends suggest that face masks and hand hygiene should be encouraged during seasonal influenza outbreaks and especially during the beginning of a pandemic when vaccines may not yet be available.

This research has several limitations. First, it is possible that participants with ILI who tested negative for influenza were infected with respiratory viruses other than influenza. Variations in ILI case definitions in surveillance studies contribute to the complexity of this issue. However, as supported by the literature [19], symptoms of cough and fever/feverishness were the two strongest predictors of confirmed influenza in our study and two symptoms constituting our ILI case definition, making it a good measure for influenza infection. Moreover, the attack rates for ILI peaked at the same time as our laboratory confirmed influenza cases (a subset of all ILI cases), suggesting that our ILI outcome followed a similar attack rates as laboratory confirmed influenza (see figure S5). Therefore, ILI cases without lab confirmed flu positivity may still have been flu cases that we were unable to detect in the lab. Since participants were only required to wear masks while in their residence hall, it is possible that transmission of infection occurred outside of the residential environment when masks were not in use. Nonetheless, students at the University of Michigan live, eat, study, and some can even take their classes within residence halls, suggesting that transmission is likely to be high in this crowded and interactive setting. In addition, we did not have the funds to include a hand hygiene only group and therefore cannot disentangle the combined effects of masks and hand hygiene. Additional limitations include our reliance on self-reported data, which may be susceptible to reporting and recall bias [20]. However, we used randomized assignment of interventions and found similarity in reported behavioral habits and hand hygiene practices across intervention and control groups at baseline, which argues against differential reporting biases. Generalizability of our study findings are limited to similar environmental settings and populations. Due to the inability to blind participants to study interventions, compliance with these interventions must be considered carefully. We observed compliance, but it was not possible to gather observational data on all participants at all times and venues.

Our study demonstrated a significant association between the combined use of face masks and hand hygiene and a substantially reduced incidence of ILI during a seasonal influenza outbreak. If masks and hand hygiene have similar impacts on primary incidence of infection with other seasonal and pandemic strains, particularly in crowded, community settings, then transmission of viruses between persons may be significantly decreased by these interventions. Masks alone did not provide a benefit, suggesting that single personal protective interventions do not protect against incidence of ILI or influenza. However, it is possible that either lack of power to detect small effects from mask use alone or that the amount of time masks were worn was not sufficient alone to provide a reduction in illness. Our timely findings regarding the efficacy of masks and hand hygiene highlight the significance of examining their impact on influenza infection within community settings.

Supporting Information

Figure S1 Reported daily average number of hand washes (log transformed) by study week. This figure shows the daily average number of hand washes (log transformed) by study week in the face mask and hand hygiene group (solid line), the face mask only group (dotted line), and the control group (dashed line). The type III fixed effects model for assessing differences over time using a week * group interaction term, was not statistically significant, $F(10, 4543) = 1.43$ and $P = 0.16$.
(TIF)

Figure S2 Reported daily average seconds of hand washing (log transformed) by study week. This figure shows the daily average time for washing hands (log transformed) by study week in the face mask and hand hygiene group (solid line), the face mask only group (dotted line), and the control group (dashed line). The type III fixed effects model for assessing differences over time using a week * group interaction term, was not statistically significant, $F(10, 4518) = 1.12$ and $P = 0.34$.
(TIF)

Figure S3 Reported daily average mask comfort rating (log transformed) by study week. This figure shows the daily average mask comfort rating (log transformed) by study week in both the face mask and hand hygiene group (solid line) and the face mask only group (dotted line). The type III fixed effects model for assessing differences over time using a week * group interaction term, was not statistically significant, $F(5, 2942) = 0.68$ and $P = 0.63$.
(TIF)

Figure S4 Reported average proportion of proper hand sanitizer use by study week. This figure shows the average proportion of respondents using the proper amount of hand sanitizer (quarter size or larger) when using hand sanitizer by study week in the face mask and hand hygiene group (black), the face mask only group (dark grey), and the control group (light grey).
(TIF)

Figure S5 Attack rate of influenza like-illness at the University of Michigan during the 2007–2008 influenza season. The attack rate of influenza and influenza like-illness among respondents and across campus.
(TIF)

Table S1 Baseline characteristics of the study population.
(DOC)

Table S2 Log reported average daily hand washing per week and P values comparing average washing in each group with face mask and hand hygiene.
(DOC)

Table S3 Log reported average wash time in seconds per week and *P* values comparing wash time in each group with face mask and hand hygiene.
(DOC)

Table S4 Log reported average face mask comfort per week and *P* values comparing comfort in the face mask only group with face mask and hand hygiene.
(DOC)

Table S5 Proportion of influenza-like illness cases who tested positive for influenza infection as determined by polymerase chain reaction (PCR).
(DOC)

Table S6 Proportion of subjects using a quarter or greater amount of alcohol sanitizer and *P* values comparing each group using the Donner and Donald chi-square test.
(DOC)

Table S7 Observational data based on the total hours of observation in each residence hall and the percentage of shifts in which participants were seen properly wearing facemasks.
(DOC)

Text S1 Additional methods and compliance measures. This text provides additional information about randomization and distribution of supplies, behavioral measures collected, additional compliance measures, laboratory methods and study attack rate.
(DOC)

Checklist S1 CONSORT checklist. This text provides the clustered CONSORT checklist for our study.
(DOC)

Protocol S1 Trial protocol. This text provides additional information about the protocol of the study.
(PDF)

References

1. Aiello AE, Coulborn RM, Aragon TJ, Baker MG, Burrus BB, et al. (2010) Research findings from nonpharmaceutical intervention studies for pandemic influenza and current gaps in the research. Am J Infect Control 38: 251–258.
2. Aiello AE, Murray GF, Perez V, Coulborn RM, Davis BM, et al. (2010) Mask use, hand hygiene, and seasonal influenza-like illness among young adults: a randomized intervention trial. J Infect Dis 201: 491–498.
3. Hayes RJ, Bennett S (1999) Simple sample size calculation for cluster-randomized trials. Int J Epidemiol 28: 319–326.
4. Cohen S, Kamarck T, Mermelstein R (1983) A global measure of perceived stress. J Health Soc Behav 24: 385–396.
5. Ohmit SE, Victor JC, Rotthoff JR, Teich ER, Truscon RK, et al. (2006) Prevention of antigenically drifted influenza by inactivated and live attenuated vaccines. N Engl J Med 355: 2513–2522.
6. Donner A, Klar N (1994) Cluster Randomization Trials: Theory and Application. J Stat Plan Inference 42: 37–56.
7. Donner A (1989) Statistical methods in opthalmology: An Adjusted chi-square approach. Biometrics 45: 605–611.
8. Donner A, Donald A (1988) The statistical analysis of multiple binary measurements. J Clin Epidemiol 41: 899–905.
9. Piantadosi S (2005) Clinical Trials: A Methodologic Perspective. New York: Wiley & Sons.
10. Allison PD (1982) Discrete-time methods for the analysis of event histories. Sociol Methodol 13: 61–98.
11. Zeger SL, Liang KY (1986) Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42: 121–130.
12. Jefferson T, Foxlee R, Del Mar C, Dooley L, Ferroni E, et al. (2008) Physical interventions to interrupt or reduce the spread of respiratory viruses: systematic review. BMJ 336: 77–80.
13. Cowling BJ, Chan K-H, Fang VJ, Cheng CKY, Fung ROP, et al. (2009) Facemasks and Hand Hygiene to Prevent Influenza Transmission in Households: A Randomized Trial. Ann Intern Med 151.
14. MacIntyre CR, Cauchemez S, Dwyer DE, Seale H, Cheung P, et al. (2009) Face Mask Use and Control of Respiratory Virus Transmission in Households. Emerg Infect Dis 15: 233–241.
15. Larson EL, Ferng YH, Wong-McLoughlin J, Wang S, Haber M, et al. (2010) Impact of non-pharmaceutical interventions on URIs and influenza in crowded, urban households. Public Health Rep 125: 178–191.
16. Canini L, Andreoletti L, Ferrari P, D'Angelo R, Blanchon T, et al. (2010) Surgical mask to prevent influenza transmission in households: a cluster randomized trial. PLoS ONE 5: e13998.
17. Cowling BJ, Zhou Y, Ip DK, Leung GM, Aiello AE (2010) Face masks to prevent transmission of influenza virus: a systematic review. Epidemiol Infect 138: 449–456.
18. Lau JTF, Griffiths S, Choi K-c, Lin C (2010) Prevalence of preventive behaviors and associated factors during early phase of the H1N1 influenza epidemic. Am J Infect Control 38: 374–380.
19. Thursky K, Cordova SP, Smith D, Kelly H (2003) Working towards a simple case definition for influenza surveillance. J Clin Virol 27: 170–179.
20. Mitchell T, Dee DL, Phares CR, Lipman HB, Gould LH, et al. (2011) Non-pharmaceutical interventions during an outbreak of 2009 pandemic influenza A (H1N1) virus infection at a large public university, April–May 2009. Clin Infect Dis 52 Suppl 1: S138–145.

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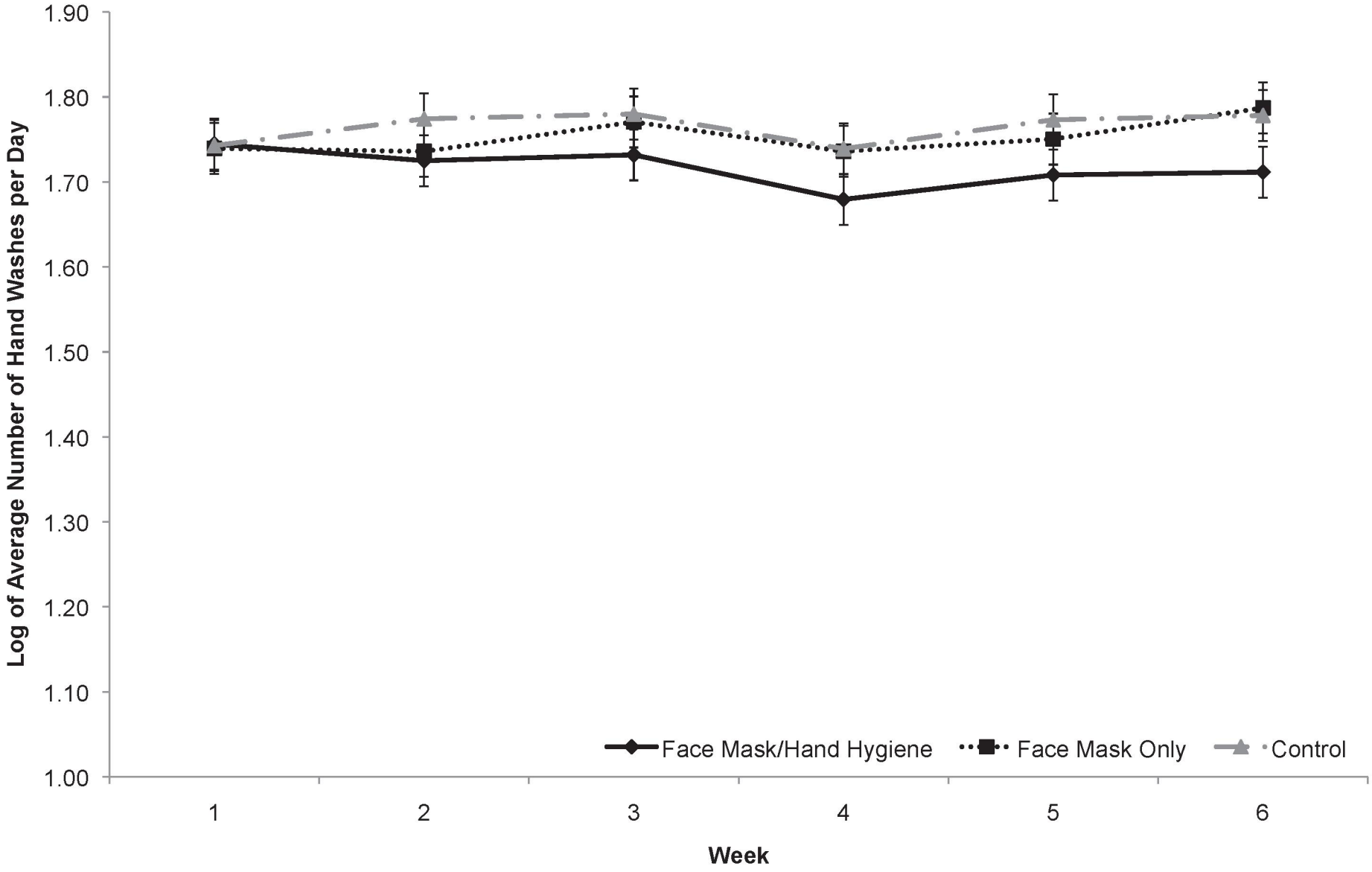
Author Contributions

Conceived and designed the experiments: AA AM. Performed the experiments: AA AM VP RC BD. Analyzed the data: AA VP BD. Wrote the paper: AA AM VP RC BD MU.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1.30
1.20
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Log of Average Number of Hand Washes per Day
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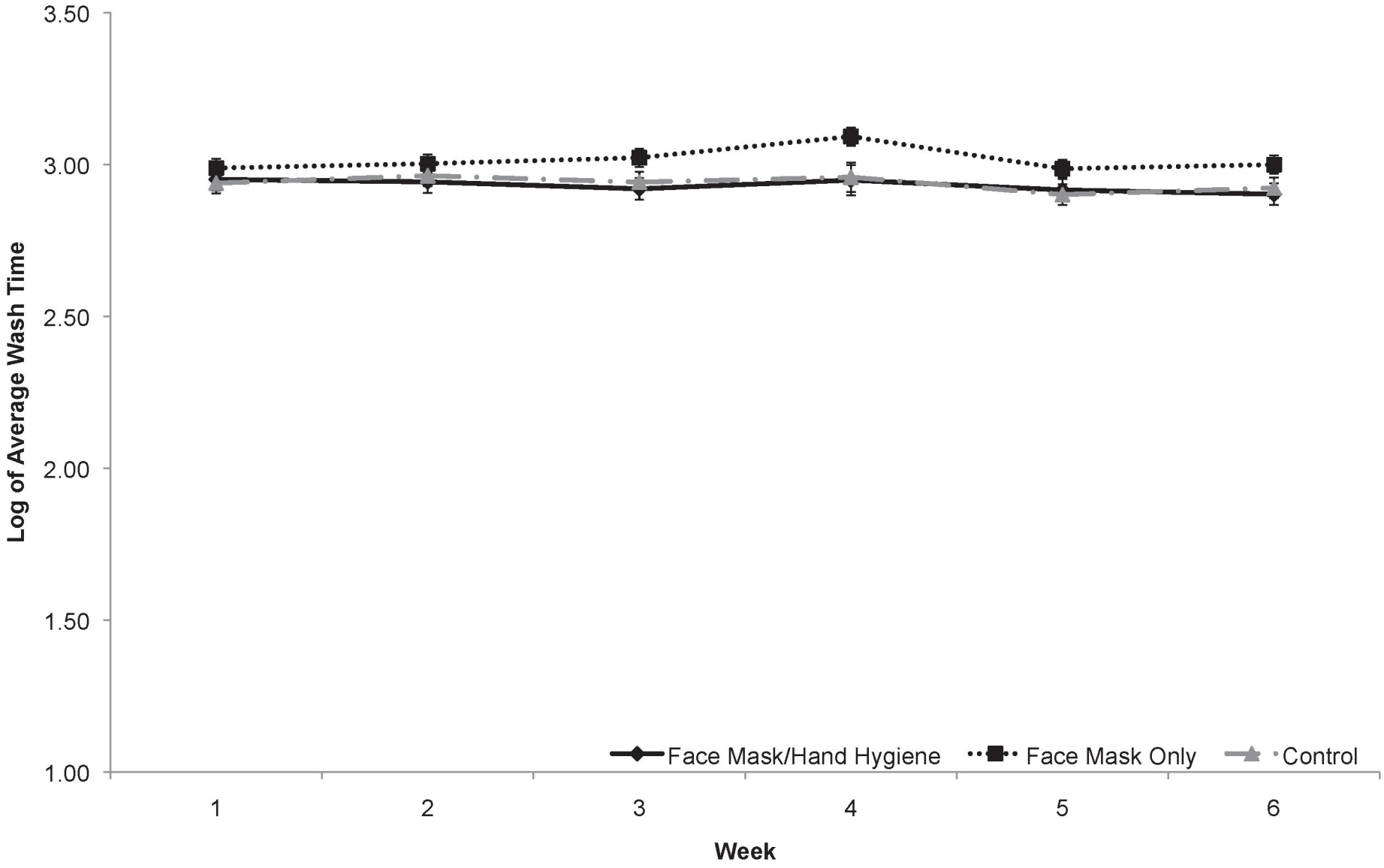
2
3
4
5
6
Week
Face Mask/Hand Hygiene
Face Mask Only
Control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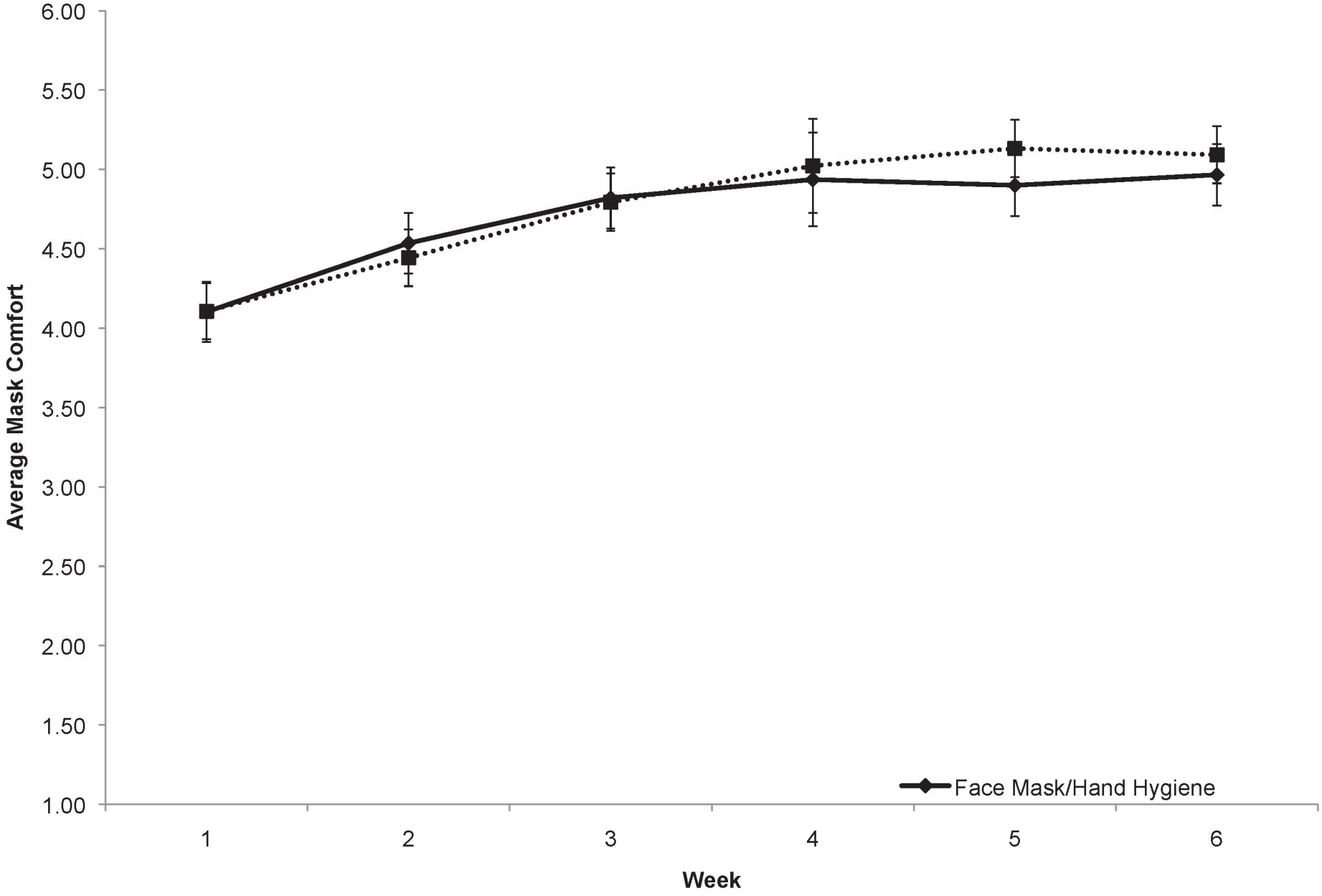
Log of Average Wash Time
Face Mask/Hand Hygiene
Face Mask Only
Control
1
2
3
4
5
6
Week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4.50
4.00
3.50
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1.50
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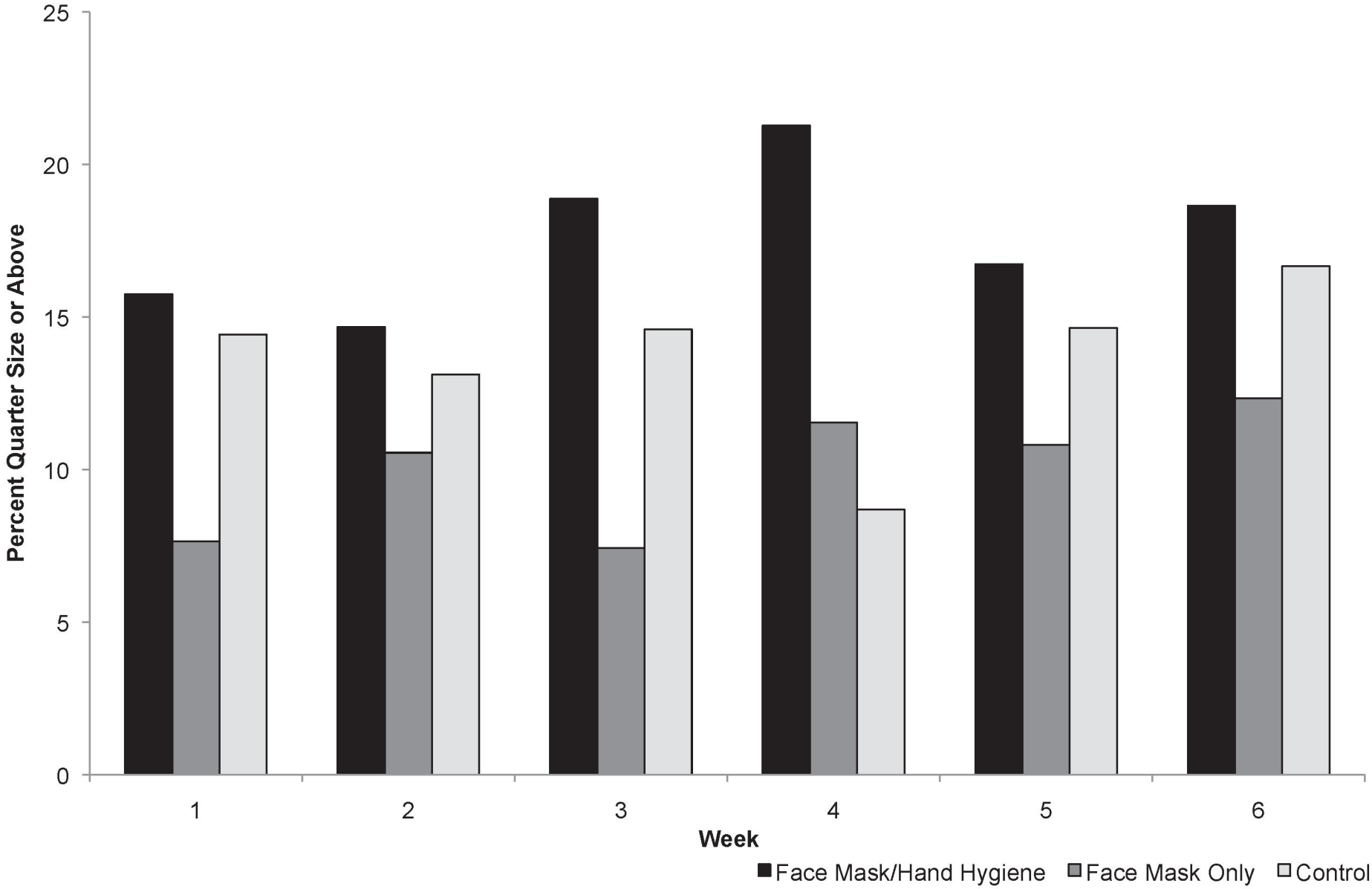
Average Mask Comfort
1
2
3
4
5
6
Week
Face Mask/Hand Hygiene


25
20
15
10
5
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Percent Quarter Size or Above
1
2
3
4
5
6
Week
Face Mask/Hand Hygiene
Face Mask Only
Control

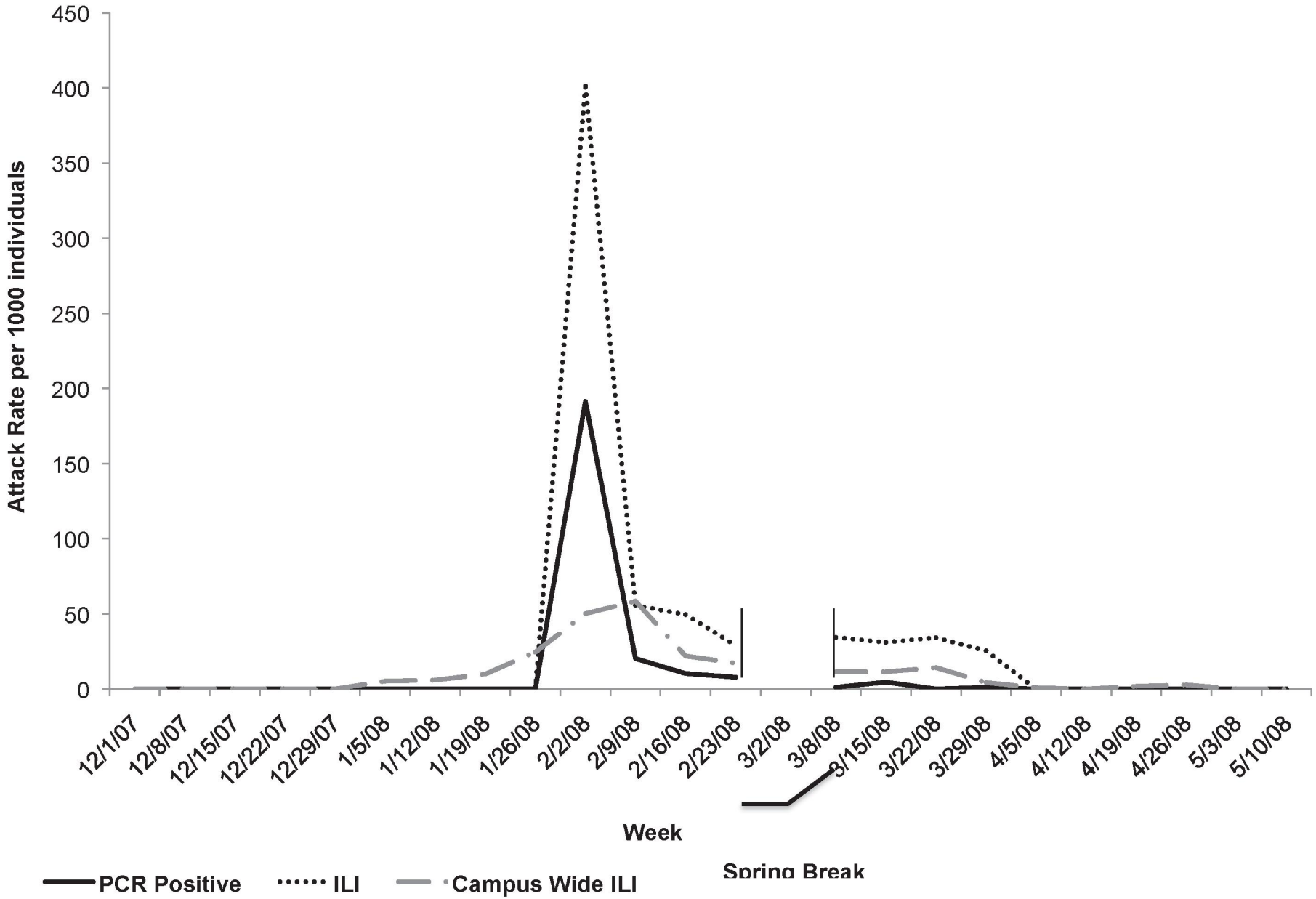
Attack Rate per 1000 individuals
450
400
350
300
250
200
150
100
50
0
12/1/07
12/8/07
12/15/07
12/22/07
12/29/07
1/5/08
1/12/08
1/19/08
1/26/08
2/2/08
2/9/08
2/16/08
2/23/08
3/2/08
3/8/08
3/15/08
3/22/08
3/29/08
4/5/08
4/12/08
4/19/08
4/26/08
5/3/08
5/10/08
Week
Spring Break
PCR Positive
ILI
Campus Wide ILI

Table S1. Baseline characteristics of the study population (N=1,111)[a]

Characteristics	Overall	ICC[b]	No. (%) of Participants: Face Mask Hand Hygien	Face Mask Only	Control	*P*[c]
Number of residence houses	37		12	13	12	
Average residence house size	29.9		29	30.1	30.8	
Total number of participants	1,111		349	392	370	
Age in years, mean (SD)[d]	18.95 (0.9)	0.11	19.01 (0.9)	18.95 (1.0)	18.90 (0.9)	0.99
Gender		0.18				0.82
Female	611 (55)		179 (52)	225 (58)	207 (56)	
Male	496 (45)		168 (48)	166 (42)	162 (44)	
Ethnicity		0.02				0.93
Hispanic or Latino	57 (5)		17 (5)	20 (5)	20 (6)	
Not Hispanic or not Latino	1014 (95)		316 (95)	360 (95)	338 (94)	
Race		0.05				0.31[e]
White	690 (64)		201 (60)	261 (68)	228 (64)	
Black/African American	102 (9)		37 (11)	29 (8)	36 (10)	
Asian	214 (20)		70 (21)	66 (17)	78 (22)	
Other[f]	68 (6)		25 (8)	26 (7)	17 (5)	
Sleep Quality		0.007				0.94
Very/Fairly Bad	237 (22)		76 (22)	85 (22)	76 (21)	
Very/Fairly Good	849 (78)		265 (78)	300 (78)	284 (79)	
Perceived Stress[g], mean score (SD)	23.14 (7.6)	0.02	23.33 (7.5)	23.06 (7.6)	23.04 (7.8)	0.99
Smoking		0.02				0.19
Current	30 (3)		8 (2)	15 (4)	7 (2)	
Non-Smoker	1055 (97)		334 (98)	369 (96)	352 (98)	
Alcohol consumption, drinks/week		0.04				0.82[h]
0	706 (66)		221 (67)	246 (65)	239 (68)	
1 to 7 drinks	174 (16)		52 (16)	62 (16)	60 (17)	
8 or more drinks	184 (18)		59 (18)	72 (19)	53 (15)	

Exercise[i]			0							0.34
Low Rate	772	(72)		241	(72)	268	(70)	263	(74)	
High Rate	300	(28)		95	(28)	114	(30)	91	(26)	
Flu Vaccine			0.001							0.46
Never	548	(52)		180	(55)	191	(51)	177	(50)	
Ever	502	(48)		147	(45)	181	(49)	174	(50)	
Recent Flu Vaccine[j]			0							0.53
Yes	179	(17)		55	(16)	59	(16)	65	(18)	
No	886	(83)		279	(84)	320	(84)	287	(82)	
Hand Washing[k]			0.02							0.67
Optimal	283	(26)		95	(27)	93	(24)	95	(26)	
Suboptimal	822	(74)		251	(73)	298	(76)	273	(74)	
Hand Sanitizer Ownership			0.03							0.54
Yes	574	(52)		192	(56)	196	(50)	186	(51)	
No	525	(48)		152	(44)	193	(50)	180	(49)	

[a]1,111 participants eligible for intention-to-treat analyses.
[b]ICC=Intracluster Correlation Coefficient, negative ICC values were set to 0.
[c]*P* Values computed using cluster-adjusted chi-square test for categorical characteristics and cluster-adjusted ANOVA for continuous characteristics.
[d]SD, standard deviation; Total of 4 participants missing age, 2 in face mask hand hygiene, 1 in face mask, 1 in control.
[e]Includes Hawaiian, Pacific Islander, American Indian, Alaskan Native, and Multi-Ethnic; 34 participants responded "decline to answer."
[f]*P* value comparing White vs. all other race groups.
[g]Total of 87 participants missing perceived stress score, 35 in face mask hand hygiene, 22 in face mask, and 30 control.
[h]*P* value comparing zero drinks per week vs. one or more.
[i]High rate defined as exercising at a very or extremely hard rate for at least 20 minutes, 3 or more times per week or exercising at an easy, medium, or hard rate for at least 30 minutes, 5 or more times per week.
[j]Flu vaccination for the 2007-2008 flu season was measured at baseline. Respondents categorized as having recently been vaccinated according to whether they said "yes" to recent vaccination on either survey.
[k]Optimal hand washing defined as washing 5 or more times per day and for at least 20 seconds.

Table S2. Log reported average daily hand washing per week and *P* values comparing average washing in each group with face mask and hand hygiene

Intervention	Average over all weeks[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Face Mask and Hand hygiene	1.72	1.74	1.73	1.73	1.68	1.71	1.71
vs. Face Mask Only[b]	1.76	1.74	1.74	1.77	1.74	1.75	1.79
		(*P* = 0.91)	(*P* = 0.81)	(*P* = 0.40)	(*P* = 0.39)	(*P* = 0.36)	(*P* = 0.10)
vs. Control	1.78	1.74	1.77	1.78	1.74	1.77	1.78
		(*P* = 0.97)	(*P* = 0.28)	(*P* = 0.30)	(*P* = 0.36)	(*P* = 0.16)	(*P* = 0.15)

[a]The change in reported average log transformed daily hand washing over the 6 week period comparing between all three study groups (week by group interaction term) using a Type III fixed effects model resulted in an $F(10, 4543)=1.43$ and $P = 0.16$.
[b]There were no statistically significant differences at any weeks comparing daily hand washing between face mask only and the control group (all $P > 0.025$).

Table S3. Log reported average wash time in seconds per week and *P* values comparing wash time in each group with face mask and hand hygiene

Intervention	Average over all weeks[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Face Mask and Hand Hygiene	2.92	2.95	2.94	2.92	2.95	2.92	2.90
vs. Face Mask Only[b]	3.01	2.99	3.00	3.02	3.09	2.99	3.00
		(*P* = 0.44)	(*P* = 0.22)	(*P* = 0.04)	(*P* = 0.04)	(*P* = 0.16)	(*P* = 0.05)
vs. Control	2.92	2.94	2.96	2.94	2.96	2.90	2.92
		(*P* = 0.81)	(*P* = 0.68)	(*P* = 0.66)	(*P* = 0.89)	(*P* = 0.76)	(*P* = 0.67)

[a]The change in reported average log transformed wash time in seconds over the 6 week period comparing between all three study groups (week by group interaction term) using a Type III fixed effects model resulted in an $F(10, 4518)=1.12$ and $P = 0.34$.
[b]There were no statistically significant differences at any weeks comparing daily hand washing between face mask only and the control group (all $P > 0.025$).

Table S4. Log reported average face mask comfort per week and *P* values comparing comfort in the face mask only group with face mask and hand hygiene

Intervention	Average over all weeks[a]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Face Mask Hand Hygiene	4.71	4.10	4.54	4.82	4.94	4.90	4.97
vs. Face Mask Only	4.77	4.11	4.44	4.79	5.02	5.13	5.09
		(*P* = 0.99)	(*P* = 0.72)	(*P* = 0.92)	(*P* = 0.84)	(*P* = 0.38)	(*P* = 0.63)

[a]The change in reported average log transformed face mask comfort over the 6 week period comparing between the two intervention groups (week by group interaction term) using a Type III fixed effects model resulted in an F(5, 2942)=0.68 and P = 0.63.

Table S5. Proportion of influenza-like illness cases who tested positive for influenza infection as determined by polymerase chain reaction (PCR)a

Characteristics	Overall, n (%)	Face Mask/Hand Hygiene	Face Mask Only	Control
Total number of ILI cases	128	31	46	51
PCR				
Positive	34 (27)	6 (19)	12 (26)	16 (31)
Negative	94 (73)	25 (81)	34 (74)	35 (69)

[a]PCR used to detect influenza A and B viruses.

Table S6. Proportion of subjects using a quarter or greater amount of alcohol sanitizer and *P* values comparing each group using the Donner and Donald chi-square test[a]

Intervention	Average over all weeks	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Face Mask and Hand Hygiene	0.17	0.16	0.15	0.19	0.21	0.17	0.19
Face Mask Only	0.10	0.08	0.11	0.07	0.12	0.11	0.12
Control	0.15	0.14	0.13	0.15	0.09	0.15	0.17
Adjusted χ^2		$P = 0.13$	$P = 0.63$	$P = 0.04$	$P = 0.16$	$P = 0.36$	$P = 0.49$

[a]P Values were calculated using the Donner and Donald chi-square test [7].

Table S7. Observational data based on the total hours of observation in each residence hall and the percentage of shifts in which participants were seen properly wearing facemasks

	Alice Lloyd		Bursley		East Quad		South Quad		West Quad	
	% of shifts[a]	Total Hours Observed	% of shifts[a]	Total Hours Observed	% of shifts[a]	Total Hours Observed	% of shifts[a]	Total Hours Observed	% of shifts[a]	Total Hours Observed
Week 1	41.2	16.5	0.0	29.1	41.7	23.2	21.4	27.2	19.2	27.9
Week 2	0.0	10.8	30.0	32.8	27.3	20.8	6.1	32.5	4.2	24.3
Week 3	4.8	20.1	32.4	42.3	12.2	39.5	15.2	45.0	4.9	40.3
Week 4	0.0	10.5	23.3	26.6	0.0	26.9	0.0	31.3	2.9	33.0
Week 5	10.0	19.4	14.8	46.0	12.5	38.6	6.0	48.1	0.0	41.2
Week 6	0.0	18.1	19.6	43.8	0.0	41.6	1.9	50.8	4.1	48.5

[a]Percentage of shifts that participants were seen properly wearing provided face masks by staff observations.

Supporting Information

I. Randomization Procedures and Distribution of Supplies. Randomization was conducted at the residence house level (n=37). Residence houses were contained within each of the five residence halls in the study (n = 5), thus ensuring that each residence hall had residence houses assigned to each intervention and control arm. Each sequence (i.e. ordering) of the intervention and control arms was assigned a value. For example, sequence (i.e. order) 1 consisted of face mask and hand hygiene; face mask only; and control. Sequence (i.e. order) 2 consisted of face mask and hand hygiene; control; face mask only, and so on. Then, each sequence was randomly selected, whereby each had an equal probability of being selected. The first residence house within the first residence hall was then selected and assigned the first value for the randomized sequence (for sequence 1, house 1 would be assigned face mask and hand hygiene), followed by the second house being assigned the sequence (sequence 1, face mask only), and the third house being assigned the final treatment in the sequence (sequence 1, control). In each residence hall, as there was greater than three houses, more than one sequence was selected and each house was drawn until all were assigned an intervention. This process ensured that each residence house had an equal probability of being assigned to the interventions or control arm and also that each residence hall would have at least one of each intervention and control groups.

The majority of participants included in the analysis (933/1,111, 84%) filled out a baseline survey and reported on baseline ILI prior to the intervention start. The proportion of participants that were allowed to enroll between January 28th to February 12th was similar across intervention (face mask only= 16%, face mask and hand hygiene = 15%) and control (17%) groups. The intervention sequence was concealed prior to the start of the intervention period.

Participants in the face mask and hand hygiene and the face mask only groups received weekly packets of mask supplies in their student mailboxes. Student signature of a packet slip confirmed receipt of mask packets. All students in the face mask and hand hygiene intervention also received hand sanitizer (2 oz squeeze bottle, 8 oz pump bottle) labeled with their study identification number. Study-staffed tables located in each residence hall offered a surplus of face masks and hand sanitizer (with exchange of used, empty bottles) to ensure a supply chain of intervention materials. As with mask packets, participants' signatures were logged to confirm receipt of surplus supplies.

II. Behavioral Measures. Questions related to hand hygiene were asked of all study participants. A variable for optimal hand washing was constructed using the CDC recommendations of washing for at least 20 seconds with soap and water and an average of five or more times per day, as reported by study subjects. Participants whose average hand washing behavior fell below 20 seconds or less than five times per day were considered to have "sub-optimal" hand hygiene and subjects reporting average values at or above 20 seconds and five times per day were considered to have "optimal" hand hygiene. Sleep quality was recorded on a 4-point scale based on quality of sleep in the previous month. Participants were categorized according to "fairly/very good sleep" and "fairly/very bad sleep". Tobacco use (yes/no) and alcohol consumption (0-1 drinks versus 2 or more drinks per week) were determined at baseline. Participants were coded as having "high" physical activity at baseline if they reported exercising hard or extremely hard for 20 or more minutes at least 3 times per week or at any exertion for at least 30 minutes 5 or more times per week. All other subjects were classified as engaging in "low" physical activity. Perceived stress was measured at baseline using a 14-item validated scale [1] with excellent internal validity.

III. Additional Compliance Measures and Analyses. In addition to compliance with the intervention materials (mask use hours and hand sanitizer frequency) described in the main text, additional compliance measures collected on weekly surveys including number of hand washes per day, duration of hand washing in seconds, levels of comfort with mask wearing, and amount of hand sanitizer used are presented here. Among participants in the facemask only and control groups, the use of personally supplied alcohol-based hand sanitizer was examined. These study groups were not provided study-associated hand sanitizer.

Compliance assessments for reported average number of hand washes and average duration of hand washing in seconds were log transformed to normalize skewed data. Mask comfort, measured from 0 being uncomfortable and 10 being comfortable, was not transformed since the distribution was normal. In order to prevent data loss due to the transformation, a value of one was added to all variables before being log-transformed. Differences between intervention and control groups in compliance were examined using a multi-level mixed model that accounted for clustering at the residence house level. Level-1 of the model accounted for changes between individuals over the course of the study period. The second level allowed for changes within individuals across the study period. Random intercepts were allowed to account for clustering at the residence house level. We adjusted the *P* value required for significance to 0.025 to account for multiple comparisons over the study period.

When comparing hand washing behavior between study groups, the facemask and hand hygiene group washed their hands 5.20 times per day (SD, 3.33) versus 5.49 times per day (SD, 3.30) in the mask only group during the study. Subjects in the control group washed their hands an average of 5.81 times per day (SD, 5.03). On the log scale, mixed model analyses showed no

statistical differences between any of the groups ($F(10, 4543)=1.43$ and $P = 0.16$) (Table S2 and Figure S1).

Subjects in the facemask and hand hygiene group washed their hands an average of 20.53 seconds per day (SD, 12.21) compared to 22.36 seconds per day (SD, 13.07) among subjects in the facemask only group. Participants in the control group washed their hands on average 20.56 seconds per day (SD, 12.68). On the log scale, the mask and hand hygiene group reported a significantly shorter duration of hand washing at weeks 3, 4 and 6 compared to the facemask only group (Table S3 and Figure S2).

On average, the face mask and hand hygiene group rated mask comfort as a 4.71 (SD, 0.21) out of 10 (comfortable) compared to 4.77 (SD, 0.20) out of 10 for the facemask only group. There were no significant differences in mask comfort between interventions at each time period, but both groups showed a gradual increase in comfort level throughout the study period (Table S4 and Figure S3).

Amount of hand sanitizer use was dichotomized into equal to or greater than the size of a quarter compared to less than a quarter size amount. A cluster adjusted chi-square test [2] was performed to examine differences in the amount of hand sanitizer used between study groups. During week 4 of the study, there was a significantly higher proportion of participants in the mask and hand hygiene intervention group using a quarter size or greater amount of alcohol-based hand sanitizer compared to the mask only and control groups (P=0.04). There were no other significant differences at other time points between study groups (Table S6 and Figure S4).

Observed Compliance. Mask compliance was also examined within residence halls via observational data recorded by trained study staff. Staff anonymously observed the number of individuals wearing masks, both correctly and incorrectly, in public areas throughout the

residence halls on a daily basis. No contact was made between study staff and students. A total of 1308 hours of observation were collected. Observational data were analyzed based on the total hours of observation in each residence hall and the percentage of shifts in which participants were seen properly wearing facemasks. Staff observed an average of 0.0007 participants properly wearing a mask for each hour of observation over the six week study period (see Table S7).

IV. Laboratory methods. Requirements for providing a throat swab sample from study participants were based on survey reported symptoms of cough plus at least one or more of fever/feverishness, body aches, or chills. The primers used for our analysis were synthesized commercially by IDT DNA (Coralville, IA) and Biosearch Technologies (Novato, CA). Additional information, including probes, cycling conditions and primers is available upon request from the authors. Following testing, samples were stored at -70 degrees Celsius.

V. Study and campus attack rates per 1,000 individuals. The PCR positive attack rate in our study was defined by the number of positive PCR samples gathered per total number of study subjects responding each week. The peak reflects the high proportion of PCR confirmed cases early in our study (see Figure S5). The ILI attack rate is defined by the number of ILI reports per total number of study subjects responding each week. Campus-wide ILI is defined by the number of weekly reported ILI cases provided by University Health Services per total number of patients seen at the University Health Services (see Figure S5). The campus-wide ILI rate reflects a much larger population than our study population. It additionally does not capture students who sought treatment outside of the University Health Service system. The spring break one-week period ending March 1, 2008 was excluded in our calculations (see Figure S5).

References:

1. Cohen S, Kamarck T, Mermelstein R (1983) A global measure of perceived stress. J Health Soc Behav 24: 385-396.
2. Donner A, Donald A (1988) The statistical analysis of multiple binary measurements. J Clin Epidemiol 41: 899-905.

CONSORT Checklist

PAPER SECTION **And topic**	**Item**	**Descriptor**	**Reported on Page #**
TITLE & ABSTRACT			
Design	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned"), *specifying that allocation was based on clusters*	Introduction
INTRODUCTION			
Background	2	Scientific background and explanation of rationale, *including the rationale for using a cluster design*	Introduction
METHODS			
Participants	3	Eligibility criteria for participants and clusters and the settings and locations where the data were collected	Methods
Interventions	4	Precise details of the interventions intended for each group, *whether they pertain to the individual level, the cluster level, or both,* and how and when they were actually administered	Methods
Objectives	5	Specific objectives and hypotheses *and whether they pertain to the individual level, the cluster level, or both*	Methods
Outcomes	6	Clearly defined primary and secondary outcome measures *whether they pertain to the individual level, the cluster level, or both,* and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors)	Methods
Sample size	7	How *total* sample size was determined *(including method of calculation, number of clusters, cluster size, a coefficient of intracluster correlation (ICC or k), and an indication of its uncertainty*) and, when applicable, explanation of any interim analyses and stopping rules	Methods
Randomization			
Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification, matching)	Methods, Text S1
Allocation	9	Method used to implement the random allocation	Methods,

concealment		sequence, *specifying that allocation was based on clusters rather than individuals and* clarifying whether the sequence was concealed until interventions were assigned	Text S1
Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups	Methods
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated	Methods
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s) *indicating how clustering was taken into account*; methods for additional analyses, such as subgroup analyses and adjusted analyses	Methods
RESULTS			
Participant flow	13	Flow of clusters and participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of clusters and participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	Results, Figure 1
Recruitment	14	Dates defining the periods of recruitment and follow-up	Methods, Figure 1
Baseline data	15	Baseline demographic and clinical characteristics of each group *for the individual and cluster levels as applicable*	Results, Tables 1-2, Table S1
Numbers analyzed	16	Number of clusters and participants (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat". State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	Results, Tables 1-4, Table S1
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group for the individual or cluster as applicable, and the estimated effect size and its precision (*e.g.*, 95% confidence interval)	Results, Tables 3-4

Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory	Results, Table 1, Text S1
Adverse events	19	All important adverse events or side effects in each intervention group	NA
DISCUSSION			
Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes	Discussion
Generalizability	21	Generalizability (external validity) *to individuals and/or clusters (as relevant)* of the trial findings	Discussion
Overall evidence	22	General interpretation of the results in the context of current evidence	Discussion

University of Michigan School of Public Health
Reducing Transmission of Influenza by Face Masks (M-FLU)

RECRUITMENT

We sought to recruit approximately 2,200 subjects and final recruitment numbers resulted in a total of 1,178 subjects.

RECRUITMENT MATERIALS

All materials are IRB approved before dissemination.

Recruitment materials such as highlighters, posters, table tents and flyers to advertise the study to students in the five residence were developed by a graphics professional. The same images were used to develop the website http://www.sph.umich.edu/mflu/ so that recruitment and enrollment were synchronized into one effort.

RECRUITMENT METHODS

Recruitment efforts are designed to comply with IRB approved methods. Research subjects are never induced to participate by means other than voluntary consent and with knowledge of the research study and its requirements. Recruiting staff are taught to avoid coercion or undue influence when recruiting and enrolling potential participants.

Participant recruitment methods included information tables in the residence halls and at festivals, sponsored television and movie nights within the residence halls, chalking on the sidewalks outside of the residence halls, and other residence hall events. Students attending sponsored events were eligible for raffled items, including sweatshirts, t-shirts, board games, snacks. Students enrolled in the study were eligible for weekly drawings for iPod mini music players. Study staff were paired with resident staff for active recruitment during dining hours, events and for the distribution of materials and supplies. Additionally, recruitment materials were placed in all students school mailboxes to provide information about the study and the recruitment process.

ENROLLMENT

The M-FLU study was open to enrollment of all participants in eligible residence halls. In year two, participants eligible for enrollment were contacted either in person or by email and given instructions for web enrollment. Potential participants were able to access enrollment forms from any computer with internet access. Using an in-house survey system, participants were supplied with a consent form, eligibility form, demographic survey, and weekly surveys containing intervention and illness information. If the participants had questions or concerns they were directed to contact the Study Coordinator either via e-mail or telephone.

ELIGIBILITY

Year two of the intervention study only required that the participants live within one of the chosen residence halls (intervention and control) and are at least 18 years of age. In addition participants with an allergy to alcohol-based hand sanitizer were asked to exclude themselves from the study. Participants must, however, be willing to wear a face mask and/or use hand hygiene on a weekly basis, and to have a throat swab specimen collected if they have signs of illness during the influenza season.

ASSIGNMENT OF STUDY ID

Participants who are eligible for participation are assigned a unique Study ID number. Participant information including but not limited to name, address, date of birth and residence hall are entered into the electronic survey system along with their corresponding Study ID.