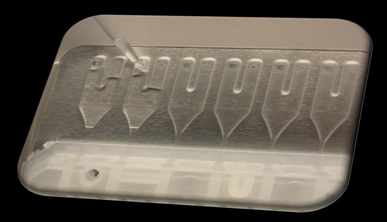
Investigation of Influenza Negative Surveillance Samples from Primary Care and Hospitalized Patients with Influenza-Like-Illness Using a Respiratory Taqman Array Card



Michael Popowich, Daryl Lamson, Henna Boolchandani, Kirsten St. George
Laboratory of Viral Diseases

NEW YORK STATE OF OPPORTUNITY. Department of Health | Wadsworth Center

Introduction

Surveillance programs utilizing a technically simple, multi-sample, sensitive and comprehensive respiratory panel, would be beneficial in determining causes of influenza-like-illness (ILI) other than influenza. This would allow for faster outbreak response and help ensure appropriate medications are being prescribed. CDC has designed a Taqman Array Card assay (TAC) with 22 respiratory targets that could enable the needed enhanced surveillance. The TAC has been shown to have a high level of sensitivity and specificity[1,2]. During the 2013-2014 influenza season, 56% of samples received at the Wadsworth Center from patients with ILI were PCR negative for both influenza A and B. In this study, we retrospectively tested influenza-negative samples using the TAC to evaluate its effectiveness as a surveillance tool. Additional goals were to ascertain differences between influenza negative inpatient and outpatient samples, identify rarely detected viruses and previously unrecognized outbreaks.

Methods

- 925 influenza-negative specimens from the 2013-2014 influenza season were tested using the respiratory TAC
  - 175 were from patients who presented with ILI at a primary care clinic
  - 750 specimens were from patients hospitalized with ILI
- Samples were extracted on a bioMerieux easyMAG: 350µl lysed and 110µl eluted
- 54ul Ambion AgPath-ID one-step RT-PCR master mix was combined with 46ul nucleic acid.
- 100ul reaction loaded onto the TAC
- TAC spun in centrifuge twice for 1min@1200RPM.
- The TAC has three control assays for each sample RP, GAPDH, and an internal control.
- Cycling was performed on an ABI ViiA 7 real-time PCR instrument.
- Samples positive in only one well, CT over 38, or with a failed control were repeated.

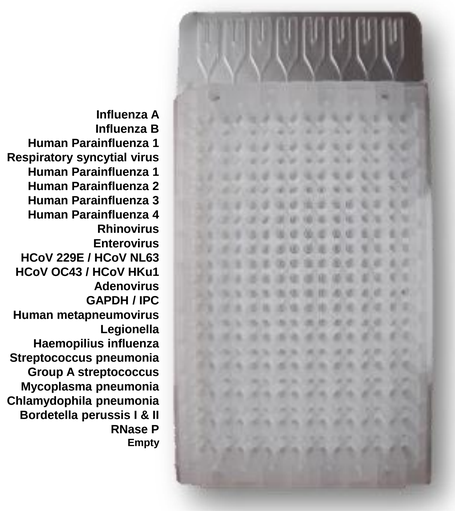


Figure 1: Diagram of the assay layout on the Taqman Array Card

Results

Pathogen Detected	Inpatient	Outpatient	Total
Negative	469	34	503
Adenovirus	3	4	7
Bordetella pertussis	0	2	2
Chlamydia pneumoniae	0	3	3
Enterovirus	5	0	5
Group A Streptoccus	7	9	16
Haemophilus influenzae	54	89	143
HCoV 229E	12	11	23
HCoV HKu1	24	8	32
HCoV NL63	12	12	24
HCoV OC43	2	4	6
Human metapneumovirus	40	18	58
Influenza A	3	0	3
Influenza B	1	0	1
Legionella	1	0	1
Mycoplasma pneumoniae	2	5	7
Parainfluenza 1	7	0	7
Parainfluenza 2	1	0	1
Parainfluenza 3	2	1	3
Parainfluenza 4	5	0	5
Respiratory syncytial virus	52	8	60
Rhinovirus	59	22	81
Streptococcus pneumoniae	60	19	79
Grand Total	821	249	1070

Table 1: Cumulative results of all pathogens detected in influenza negative outpatient and inpatient samples.

Overall positivity rate: Outpatient = 80.6% Inpatient= 37.5%

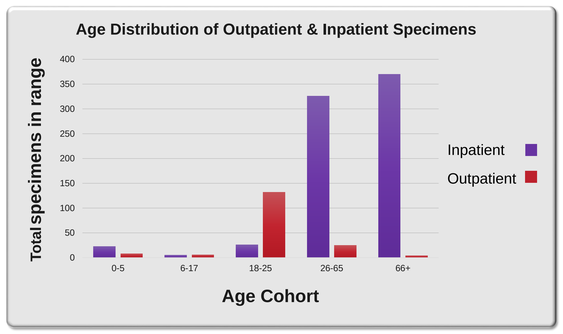


Figure 2: Age distribution of outpatient and inpatient samples tested in the study.

Large demographic difference between two surveillance programs. The majority of outpatients samples came from college students while most impatient samples came from the oldest age cohorts.

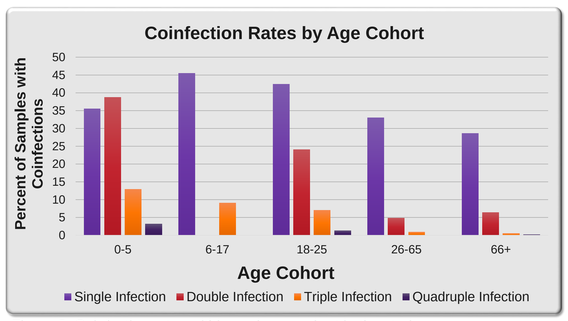


Figure 3: Coinfection rates within each age cohort in the study.

Results Continued

Virus	Positives	# Bacterial Coinfections	Percent Coinfected
Rhino	81	28	34.6
RSV	60	23	38.3
hMPV	58	13	22.4
HKu1	32	8	25.0
229E	23	7	30.4
NL63	24	6	25.0

Table 2: Bacterial coinfection rates with frequently detected viruses.

In the 0-5 age cohort, double infections were more common than single infections

Coinfection rate for the 18-25 age cohort was 32.4%: much higher than the 5-7% rate for the older age cohorts.

Frequently detected viruses had statistically similar rates of bacterial coinfection. P value = 0.0604

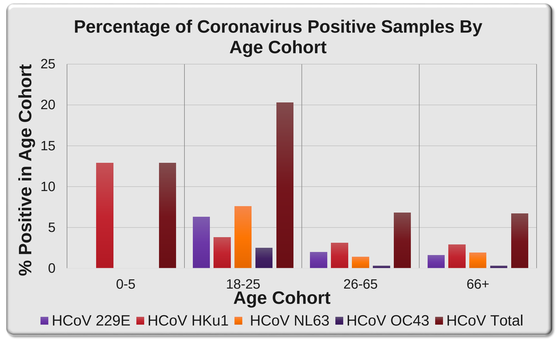


Figure 4: Coronavirus detection rates in each of the age cohorts in the study

Coronaviruses were detected in 85 of the 925 samples

HKu1 has never previously been detected in the laboratory during clinical testing.

The 18-25 year-old cohort had a 20.3% positivity rate for coronaviruses, while older age cohorts had 6-7% positivity rate.

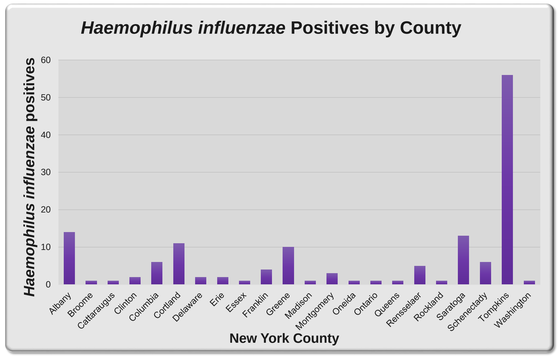


Figure 5: Number of *Haemophilus influenzae* positives by county.

52.8% of influenza negative samples tested from Tompkins county were positive for *H. influenzae.*

Conclusions

- The discrepancy between positivity rates in primary care and hospitalized patients was surprising. However, primary care patients were mostly young adults while hospitalized cases were in the oldest age groups. Senior adults may be more likely to be hospitalized for respiratory symptoms with an etiology caused by conditions other than infectious diseases.
- The 18-25 age cohort was comprised mostly of college students which had much higher coinfection rates than the older age cohorts. Living in a dormitory setting may result in an increased chance of being exposed to multiple pathogens.
- The low occurrence of adenovirus, enterovirus and parainfluenza virus infections compared to coronaviruses was surprising. Coronaviruses appear to circulate during respiratory season at a high level in association with symptomatic disease, even though they may often go undetected due to the capabilities of many routinely used diagnostic assays.
- 3 of the 5 enterovirus positive samples were coinfected with a high titer rhinovirus. These rhinoviruses are likely cross-reacting in the enterovirus assay.
- All 4 influenza positive specimens had high CT values, indicating a low viral load in the specimen. Viral load near the limit of detection for the influenza real-time RT-PCR assay may explain why the initial influenza testing was reported as negative.
- 66 samples required repeat testing due to either high CT values or a positive result detected initially in only one replicate. Among these samples, 34 generated subsequent positive results in at least one well and were considered positive for the target. Samples that were negative upon repeat testing were considered negative.
- Overall, the TAC platform performed extremely well for this type of analysis. Previous studies have demonstrated good sensitivity, specificity and reproducibility profiles for the system and the additional data from this study supports its use for broad respiratory disease surveillance.

Acknowledgements

- The Authors would like to thank APHL and CDC for funding the study and providing the Taqman Array Cards and reagents.

References

1.Kodani M, Yang G, Conklin LM, Travis TC, Whitney CG, Anderson LJ, Schrag SJ, Taylor TH Jr, Beall BW, Breiman RF, Feikin DR, Njenga MK, Mayer LW, Oberste MS, Tondella ML, Winchell JM, Lindstrom SL, Erdman DD, Fields BS. Application of TaqMan low-density arrays for simultaneous detection of multiple respiratory pathogens. J Clin Microbiol. 2011 49: 2175-82.

2. Harvey JJ, Chester S, Burke SA, Ansbro M, Aden T, Gose R, Sciulli R, Bai J, DesJardin L, Benfer JL, Hall J, Smole S, Doan K, Popowich MD, St George K, Quinlan T, Halse TA, Li Z, Pérez-Osorio AC, Glover WA, Russell D, Reisdorf E, Whyte T Jr, Whitaker B, Hatcher C, Srinivasan V, Tatti K, Tondella ML, Wang X, Winchell JM, Mayer LW, Jernigan D, Mawle AC. Comparative analytical evaluation of the respiratory TaqMan Array Card with real-time PCR and commercial multi-pathogen assays. J Virol Methods. 2016 228: 151-7.