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Panther Fusion® Respiratory Virus Assays for the detection of influenza and other respiratory viruses☆

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ABSTRACT

Background: Nucleic acid amplification tests (NAATs), such as PCR, are preferred for respiratory virus testing, due to superior diagnostic accuracy and faster turnaround time. Panther Fusion® Respiratory Assays (Fusion), which includes FluA/B/RSV (FFABR), Paraflu and AdV/hMPV/RV, offers a modular approach to syndromic testing on a fully automated platform while improving gene targets and expanding the test menu.
Objectives and study design: We evaluated Fusion using 275 consecutive nasopharyngeal specimens previously used in an analysis of five PCRs, as well as 225 archived specimens.
Results: Of the combined 500 specimens, 134 were positive for influenza A (FluA), 54 for FluB, 65 for RSV, 64 for parainfluenza (PIV), 24 for adenovirus (AdV), 21 for humanmetapneumovirus (hMPV), and 40 for rhinovirus (RV) with Fusion. Of the positive samples Fusion correlated with historical results for all but one, despite multiple freeze-thaws cycles of this collection. Fusion was positive for an additional 33 samples, including 11 FluAs, 7 RSVs, 3 PIV3s, 3 AdV, 6 hMPV and 3 RVs. These samples were retested with corresponding Prodesse (Pro) assays using quadruple sample volume. This resolver test confirmed Fusion results for an additional 4 FluAs, 4 RSVs, 1 PIV3 and 3 AdVs. The sensitivity and specificity ranges of Fusion were 99–100% and 98–100%. Limit of detection (LOD) analyses were performed on a variety of Flu isolates. The LODs ranged from 2.69 to 2.99 log copies/ml and demonstrated superior LOD as compared to previously published data for some assays or to concurrent analyses with two new commercial tests.

1. Background

Respiratory tract infections (RTI) are common and are associated with significant health burden. The major viral agents of RTI include FluA/B, RSV, hMPV, PIV1-4, AdV, and RV. The spectrum of diseases associated with viral infection of the upper and lower respiratory tract include the common cold, otitis media, influenza-like illness, croup, bronchiolitis and pneumonia; all of which can be caused by any one of these viruses, leading to diagnostic limitations based on symptoms alone.

Rapid identification is important for both therapeutic and infection control purposes. Traditional rapid diagnostics, such as immunoassays, produce quick results and are simple to perform but have sub-optimal sensitivity (Reviewed in [1]). NAATs, which are rapid and have enhanced sensitivity, are considered the method of choice by many and are recommended by IDSA Guidelines [2–4]. However, performance differences have been observed among commercial NAATs, particularly after 2014 when sequence divergence in the matrix gene of A(H3N2) viruses emerged [5–7]. Especially problematic was a C163 T mutation that was first observed among 3C.2a clades of A(H3N2) [8–10]. Commercial assays with significantly reduced sensitivities after the C163 T mutation emerged, included Prodesse ProFlu + (PFlu) and Xpert® Flu (Xpt).

The Panther Fusion® Respiratory Virus assays on the fully-automated Panther Fusion® system include the Flu A/B/RSV, Paraflu, and AdV/hMPV/RV (Table 1). This new system from Hologic has redesigned amplification reactions as compared to Pro. The FluA component still targets the matrix gene, but it uses a dual target approach with multiple probes for added redundancy to help safeguard against genetic drift. Both FluB and RSV now target the matrix gene and the AdV hexon gene target is designed to detect all AdV genotypes. The gene targets for hMPV and PIV 4 are the nucleocapsid genes, while those for PIV 1–3

☆ Panther Fusion® Respiratory Virus Assays excellent performance with a challenging sample population. twitter handle @KathyStellrk

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Table 1
Abbreviations used for test methods.

Abreviation	Description
Fusion	Panther Fusion® Respiratory Assays, the complete set of 3 assays for respiratory virus
FFABR	Panther Fusion® FluA/B/RSV for the detection of influenza A, B and respiratory syncytial virus
Paraflu	Panther Fusion® Paraflu for the detection of parainfluenza, types 1-4
AdV/hMPV/RV	Panther Fusion® AdV/hMPV/RV for the detection of adenovirus, human metapneumovirus and rhinovirus.
Pro	Prodesse Respiratory Assays, the complete set of 5 assays for respiratory virus
PFlu	Prodesse ProFlu + for the detection of influenza A, B and respiratory syncytial virus
PFAST	Prodesse ProFAST + for influenza A strain typing
ProAdV+	Prodesse ProAdV + for the detection of adenovirus
Pro_hMPV+	Prodesse ProHMPV + for the detection of human metapneumovirus
ProPara+	Prodesse ProPara + for the detection of parainfluenza, types 1-3
Xpt	Xpert® Flu for the detection of influenza A and B, with an H1 call-out
RP	FilmArray Respiratory Panel 1.7
RP2	FilmArray Respiratory Panel 2
cIAB	cobas® Influenza A/B test

and RV are the hemagglutinin-neuraminidase region and the 5′ UTR, respectively. This test system also expands the menu of virus detected with the inclusion of a RV and PIV4.

2. Objectives

This study looks at the performance of the Panther Fusion® Respiratory Virus panels on a collection of samples that were previously analyzed with five other respiratory virus assays [7], to effectively enable a comparative analysis. This population set was also highly representative of the A(H3N2) clade (3C.2a), truly challenging any FluA assay.

3. Study design

3.1. Specimens

Clinical specimens included 275 consecutive nasopharyngeal swab specimens, in 3 ml of viral transport medium, received into the laboratory for the detection of respiratory viruses during a 2-week period in the winter of 2015 (age range 22 d to 93 yr., median 25 yr., 45% pediatric cases, Table S1). These specimens were previously used in a prospective analysis of PFlu and PFAST (Hologic, San Diego, CA), FilmArray Respiratory Panel 1.7 (RP, BioFire, Salt Lake City, UT), and cobas® Influenza A/B test (cIAB, Roche Diagnostics, Indianapolis, IN) specifically for the detection of FluA. Subsequently, Xpt (Cepheid, Carlsbad, CA) was analyzed retrospectively. Specimens were stored at -80 °C, after original testing with PFlu/PFAST (49%) or RP (51%), thawed for cIAB and RP or PFAST testing, frozen at -80 °C, thawed and frozen for Xpt testing. Some were frozen and thawed additional times for previous discrepancy analysis. They were again thawed for testing by Fusion. Selected archived samples positive for respiratory viruses (n-225), previously tested by various combinations of NAATs, were analyzed.

Clinical specimens containing low Ct values on Fusion (presumably high titers) of RSV, PIV1, PIV3, hMPV, AdV, and RV were serially diluted for endpoint comparison of Fusion with the appropriate Pro assays (PFLU, PFAST, ProParaflu+, ProAdeno+, or Pro_hMPV+, Hologic) except for the RV sample which was compared with RP2.

3.2. Viruses

FluA isolates obtained through the NYS DOH Proficiency Testing program were classified based on HA sequences and included A/California/7/2009(H1N1)pdm09-like (09H1N1), A/Perth/16/2009(H3N2)-like (Perth), A/Texas/50/2012(H3N2)-like (Texas) HA gene, and B/Massachusetts/2/2012-like (FluB). Texas was later shown to be a chimeric virus with an A/Hong Kong/5738/2014-like M1 gene [7]. Texas is also A/New_York/04/2014 (EPI_ISL_157766) in the GISAID database (personal communication with Jennifer LaPlante, NYS DOH Wadsworth Center). A/Switzerland/9715293/2013(H3N2)-like (Swiss) and A/Indiana/09/2012(H3N2v)-like were gifts from Jennifer LaPlante and Judith Lovchik, Indiana State Department of Health, respectively. Viral stocks were serially diluted 1:10 in VTM and tested in quadruplicate for LOD analyses. Viral nucleic acid concentration determinations were based on quantified control viral RNA (Hologic) using either PFlu or PFAST, depending on strain-based amplification efficiency.

3.3. Nucleic acid extraction and amplification

Fusion Assays (Hologic) were performed on the fully automated Panther Fusion® with continuous, random access. This instrument utilizes universal nucleic acid extraction and PCR chemistry. The assay specific reagents are available in ready to use reagent cartridges. Initially, 500 µL of specimen was added to a Panther Fusion Specimen Lysis Tube containing 750 µL buffer and 360 µL of the mixture is used for an extraction. The nucleic acid was subsequently eluted into 50 µL and 5 µL were amplified for an effective sample volume tested of 14.4 µL.

LOD studies were expanded to newer tests kits, RP2 and Cepheid Xpert® Xpress Flu (Xpress), both of which have been marketed as having improved sensitivities among currently circulating strains of influenza. Testing was performed in accordance with the manufacturer's package insert.

3.4. Statistical analysis

Specimens were considered true positive (TP) using a composite reference standard (CRS) defined as positive with previously published results [11]. Samples equivocal for FluA with RP were considered positive by that test method. The resolver test involved a modification of Pro assays to enhance the analytical sensitivity. Specifically, viral RNA was extracted from 0.4 ml of specimen (twice the normal volume) using the easyMAG extractor (bioMérieux, Durham, NC) and eluted to a volume of 25 µL. RNA extracts, 5 µL (effective sample volume tested of 80 µL), were amplified with the appropriate Pro assay on SmartCyclers (Cepheid). Sensitivities, specificities, and confidence intervals (CI) were determined using Microsoft Excel 2016 (Redmond, WA) [11]. Probit analyses for the limit of detection with a 95% probability of detection were performed using SPSS version 8.0 (IBM, Armonk, NY).

Table 2
Fusion performance against historical data for FluA detection with the 275 consecutively collected specimens between 1/26/15 and 2/9/15.

n	TP[a]	FFABR	cIAB	PFAST	RP	Xpt	Co-Infections or other viruses
51	+	+	+	+	+	+	1 RSV & 3 RV
5	+	+	+	+	+	–	
4	+	+	–	+	+[b]	–	1 RSV & Adv
1	+	+	–	+	–	+	
2	+[c]	+	–	+	–	–	1 RSV & Adv
2	+	+	+	–	–	–	
1	+	–	–	–	+	–	
4	+[d]	+	–	–	–	–	
6	–	+	–	–	–	–	1 hMPV
199	–	–	–	–	–	–	multiple other viruses[e]

[a] True positive.
[b] 2 RP positive FluA, but not typed, and 1 RP FluA equivocal.
[c] Cells with yellow highlight were previously considered to be true negative (TN) in previous study (positive by one test only) [1].
[d] True positive after discrepancy analysis.
[e] Positive for multiple other viruses, including RSV (55), hMPV (8), PIV2 (1), PIV3 (10), ADV (7) & RV (18). Total co-infections include 3 triple infections (2 H3N2, RSV & AdV, 1 RSV, AdV and RV) and 13 dual infections (1 H3N2 & RSV, 3 H3N2 & RV, 1 H3N2 & hMPV, 1 RSV & PIV3, 2 RSV & AdV, 4 RSV & RV, 1 PIV3 & RV).

4. Results

4.1. Performance with consecutive clinical samples

The performance of Fusion was first evaluated using 275 consecutive nasopharyngeal specimens collected during the peak of the influenza season in January-February 2015. This sample set was previously used in a prospective analysis for cIAB, PFlu/PFAST, RP and Xpt. The incidence of FluA in this population was previously considered to be 24%, exclusively A(H3N2), with 66 true positive cases. Even though this collection of specimens has been used in multiple studies and has gone through multiple freeze-thaws cycles, FFABR was FluA positive for 65 of the TP cases (Table 2). The one false negative sample was previously positive by RP only. FFABR was positive in an additional 10 samples which were previously negative by other methods.

The 10 additionally positive samples were analyzed with a resolver test which involved analyzing quadruple the initial specimen volume for testing by PFAST, resulting in an additional 4 T P specimens. As a result of reclassification after resolver testing, the incidence of FluA in the sample set was now 25%. The sensitivity and specificity of FFABR for the detection of A(H3N2) circulating in 2015 were 98.7% and 97.1%.

This sample set was also positive for multiple other viruses, including RSV (55), PIV2 (1), PIV3 (10), AdV (7), hMPV (8), and RV/EV (18) and 118 specimens were negative for any virus. Total co-infections in this set included 3 triple infections (2 H3N2, RSV & AdV, 1 RSV, AdV and RV) and 13 dual infections (1 H3N2 & RSV, 3 H3N2 & RV, 1 H3N2 & hMPV, 1 RSV & PIV3, 2 RSV & AdV, 4 RSV & RV, 1 PIV3 & RV).

4.2. Performance with additional respiratory viruses

To expand the analysis of Fusion with other respiratory viruses, 225 archived, respiratory virus positive samples were evaluated. The viruses included in this sample set were A(H1N1)pdm09 (49), A(H3N2) from 2009 (5), seasonal A(H1N1) from 2009 (4), FluB (54), RSV (7), PIV1 (15), PIV2 (3), PIV3 (16), PIV4 (17), AdV (17), hMPV (7), and RV/EV (20), as well as 25 samples negative for all Fusion detectable viruses but positive for either coronaviruses or atypical bacteria (Table 3). Total co-infections in this population include 7 dual infections (2 FluB & RSV, 1 FluB & AdV, 2 PIV & AdV, 1 PIV & RV, 1 RV & *M. pneumonia*). From the cumulative 500 specimens, Fusion results were 134 positive for FluA,

Table 3
Fusion performance against historical data for respiratory virus detection with the 225 archived samples.

True Pos	*n*	FFABR	Pro[a]	RP	cIAB	Xpt	Co-Infections
H1N1pdm09[b]	46	+	+	+[c]	+	+[d]	
H1N1pdm09	1	+	+	+	+	–	
H1N1pdm09	2	+	+	–	+	+	
H3N2	5	+	+	+[e]	+	+	
sH1N1[f]	4	+	+	+[e]	+	+	
FluB	25	+	+	+	+	+	2 RSV
FluB	1	+	+	+	+	–	
FluB	2	+	+	–	+	–	
FluB	2	+	–	+	+	–	
FluB	13	+	+	na[g]	na	na	
FluB	11	+	na	+	na	na	1 AdV
RSV	2	+	na	+	na	na	
hMPV	6	+	na	+	na	na	
PIV1	15	+	na	+	na	na	
PIV2	3	+	na	+	na	na	
PIV3	16	+	na	+	na	na	1 AdV & 1 RV
PIV4	17	+	na	+	na	na	1 AdV
AdV	1	+	+	–	na	na	
AdV	11	+	na	+	na	na	
RV	16	+	na	+	na	na	1 *M. pneumonia*
True Neg	1	–	–	PIV1	na	na	
True Neg	1	FluA	–	–	na	na	
True Neg [h]	21	–	–	–	na	na	
Unknown [i]	3	–	na	RVEV	na	na	
Total	225						

[a] Virus specific Prodesse test.
[b] A(H1N1)pdm09.
[c] 6 were equivocal for FluA with RP.
[d] 1 repeatedly produced an error with Xpt.
[e] 1 positive for FluA with RP but not typed.
[f] Seasonal A(H1N1) circulating in 2009 prior to the pandemic gNot analyzed.
[h] True negative for organisms tested for with Fusion. Other organisms detected by RP were 11 coronaviruses and 8 atypical bacteria.
[i] True status unknown, discrepant between only two methods for detecting RV. Negative for EV with lab developed test.

54 for FluB, 69 for RSV, 65 for PIV, 27 for AdV, 21 for hMPV, and 41 for RV. Fusion correlated with all historical positive results except 6 samples negative with Fusion RV but positive for EV/RV by RP. These six samples were tested by our lab developed assay for EV and were positive, excluding them as false negative cases. Fusion had 29 positive test results not detected by another method, including 1 more FluA, 7 RSV, 3 PIV3, 3 AdV, 6 hMPV and 3 RV. These samples, except the 3 positive for RV, were analyzed by the appropriate resolver tests, which confirmed Fusion results for 4 RSV, 1 PIV3 and 3 AdV. The sensitivity of Fusion ranged from 99 to 100% for the various viruses and the specificity ranged from 98% to 100% (Table 4).

4.3. Limit of detection

Because previous studies demonstrated FluA assay performance variations can be strain associated, we performed LOD analyses with six isolates of Flu. We also included RP2 and Xpress in this analysis as these assays were new to market and considered to have improved viral strain coverage. Fusion demonstrated excellent analytical sensitivity with low LODs ranging from 2.69 to 2.99 log copies/ml (Table 5). The LODs were highly consistent across the strains and clades of FluA. The assay was highly reproducible with coefficient of variances ranging from 0.5 to 4.2% across the dilutions (data not shown). RP2 and Xpress demonstrated improved analytical sensitivity and consistency as compared to the manufacturer's previous test systems. However, the LODs were still relatively higher, particularly with newly circulating A(H3N2) subclades.

To assess differences in analytical sensitivity for the other Fusion targets versus Pro or RP, clinical specimens containing RSV, PIV1, PIV3,

Table 4
Fusion performance against historical data for respiratory virus detection in total collection of 500 samples.

virus	TP	Fusion TP	Fusion FP	TN	Fusion TN	Sens	Spec
H1N1pdm09	49	49		451	451	100%	100%
H3N2	75	74	7	418	418	99%	98%
sH1N1	4	4		496	496	100%	100%
FluB	54	54		446	446	100%	100%
RSV	66	66	3	435	435	100%	99%
hMPV	15	15	6	479	479	100%	99%
PIV1	15	15		485	485	100%	100%
PIV2	4	4		496	496	100%	100%
PIV3	27	27	2	472	472	100%	100%
PIV4	17	17		483	483	100%	100%
AdV	24	24		476	476	100%	100%
RV	38	38	3	459	459	100%	99%
All Viruses	391	390	21	146[a]	135	100%	87%

[a] Samples with no virus detected (total true results among 500 samples was 525).

hMPV, AdV, and RV were serially diluted for endpoint comparison. Fusion was positive for an additional 10-fold dilution for RSV, PIV3, and AdV and two 10-fold dilutions for hMPV, while the endpoint positivities were similar between Fusion and Pro or RP for PIV1 and RV (data not shown).

5. Discussion

Since their introduction, there have been numerous studies regarding the performance of NAATs for respiratory virus detection, whether multianalyte panels or Flu or Flu/RSV specific, as various systems came on the market to fill individual niches and needs (reviewed in [12]). Prodesse assays had always been thought of as a necessary comparator system, if not the gold standard [13–18]. But FluA target failures became common with A(H3N2) subclades that emerged in 2013 [7,9]. In fact, since 2014 most all circulating A(H3N2) viruses had M1 gene mutations which significantly affected the sensitivities of PFlu and Xpt. As a result, many manufacturers modified their systems to enable expanded clade coverage. Indeed, Hologic uses influenza redundancy in the design of the Panther Fusion FluA/B/RSV assay.

Our sample populations offered a unique opportunity to challenge the performance of Fusion with A(H3N2) samples containing the highly problematic C163 T mutation in the M1 target region [6,7], as well as evaluate the performance among historic clades and strains of virus. Furthermore, many of these samples have been used in previous analyses of other systems, effectively enabling a multisystem analysis. In a recent study by Banerjee et al. [19], FFABR was compared to 5 FluA/B PCRs, including cIAB and RP. However, this study involved selected pediatric specimens collected over five respiratory virus seasons, as opposed to all specimens received within a period of high incidence, which is a better challenge of test sensitivity. In addition, pediatric cases are known to have higher viral titers, which also poses less of challenge with regards to test sensitivity. As a result, the sensitivities of all assays with this contrived population were comparable.

Interestingly, in our study, Fusion was positive for all but one virus previously detected in these samples, even though this collection has been used in multiple studies and has gone through multiple freeze-thaw cycles. There were 12 additional Fusion positive samples, which were confirmed true positive by analysis on Pro using higher sample input volumes. Indeed, the need for more sensitive methods for discrepancy analysis for Fusion has been demonstrated by others [20].

This leaves 21 samples not confirmed by a different method. It is not clear if this phenomenon is due to the greater analytic sensitivity of Fusion or due to true false viral detection. The superior analytical sensitivity was demonstrated in the LOD and endpoint dilution studies, in addition to the fact that the test performed exceptionally well with highly compromised samples in terms of specimen handling for maintaining viral RNA titers. Indeed, it appears the FP samples had much lower virus titers, as demonstrated by Ct values (Supplemental figure 1); hence, a system with superior analytical sensitivity would demonstrate better performance with such specimens. Furthermore, the additional positive viruses were only observed in samples collected during periods of high viral prevalence, for example during the 2-week period of 25% FluA incidence. False positive detection or signal would be a random event and be equally evident at periods of low viral incidence. It is also important to point out that 3 F P samples were due to RV and unfortunately, we only had one other system available in our lab to test for RV. Hence, there was no true referee of the difference in the two systems.

Although this manuscript was not a reanalysis of cIAB, Pro, RP, and Xpt, the reclassification of some samples as TP would result in changes in the sensitivities and specificities of these other test systems. In particular, for the consecutive samples from 2015, the specificities of these other assays would improve to 100% from our previous publication [7]. However, the sensitives would decrease by 4 to 10%. It is also important to point out the choice of reference method in the absence of a gold standard has a significant impact on test performance. If this study involved submission of a diagnostic test to the Food and Drug Administration (FDA), a majority criteria would be required for a non-reference standard and a resolver test would not be allowed for the determination of positive and negative agreement, so as to prevent the introduction of bias in favor of the new test [21]. However, it is also known that this approach is biased against a new test with superior analytical sensitivity and in such situations a CRS is recommended [11].

Another limitation with this study is the fact that the resolver method does not test another target. However, our need was to have a resolver that more closely matches the LOD of the Fusion. Having an assay with a low LOD is not an easy feat; hence, it's not easily accomplished with another gene target for the sake of a less biased resolver. Pro was chosen as the resolver because 1) it had LOD lower than any of the other commercial assays [10] and 2) it can be modified to increase the effective testing volume. Likewise, Sanger sequencing which is often used as a resolver, it doesn't have a low LOD. For example, HIV genotyping can only be performed on patients with viral loads of 1000 copies/ml. Indeed, our lab was only able sequence the influenza matrix gene for samples with low Ct values [10].

Besides the exquisite sensitivity seen with Fusion, the system has numerous other benefits (Table 6). It is a fully automated, high throughput system with on-demand testing capabilities. The strategy

Table 5
Limit of Detection (estimated log copies/ml) for Fusion, RP2 and Xpress.

Virus	Strain (*source)*	H3N2 Clade	Fusion	RP2	Xpress
H1N1pdm09	A/California/07/2009-Like *(NYS-PT)*	na	2.77	2.85	3.08
sH3N2	A/Perth/16/2009-Like (*NYS-PT)*	1	2.69	3.37	2.69
sH3N2	A/Switzerland/9715293/2013-Like *(NYS-Coll)*	3C.3a	2.75	4.57	3.58
sH3N2	A/Texas/50/2012-Like (*NYS-PT)*[a]	3C.2a	2.96	3.87	3.48
H3N2v	A/Indiana/09/2012-Like *(IS)*	vH3	2.82	2.83	2.84
FluB	B/Massachusetts/02/2012-Like (*NYS-PT)*	na	2.99	3.86	3.64

[a] Chimeric isolate with A/Texas/50/2012-Like HA gene and A/Hong Kong/5738/2014-like M1 gene.

Table 6
Advantages and Disadvantages of the Panther Fusion System.

Advantages	Disadvantages
Higher clinical sensitivity	Large footprint
Low limit of detection	Time to first result = 2.4 hours
Low percentage of invalid results (0.3%)	Refrigeration needed for assay cartridge storage
Broad range of respiratory viruses detected	Lacks subtyping for influenza A strains
Modular approach to syndromic testing, the same extract can be used for 1 to 3 respiratory assays	The storage capacity for the reaction tubes does not meet the needs of the maximum specimen capacity
Highly automated with little hands on time	Controls cannot be tested prior to specimen testing; at least one specimen or a blank must be tested concurrently
On demand testing	
Process up to 335 respiratory tests in 8 hrs.	
Multiple PCRs and TMAs on board; can run up to 12 different protocols at one time	
Reagents stable on-board for up to 60 days.	
Reagent and consumable tracking with advance warning if more is needed and when it is needed	

used by Hologic is for a modular approach to syndromic testing, with 3 respiratory assays for 10 targets. However, it is lacking subtyping for FluA strains. Another plus is that the reagents are stable on-board for up to 60 days; however, refrigeration is needed for assay cartridge storage.

In summary, Fusion offers exquisite sensitivity for the detection of respiratory viruses on a fully automated platform. There are many options for respiratory virus testing, each with their own niche. Fusion appears to be the leader with regards to sensitivity, protections against genomic drift, while maintaining high throughput and minimal hands on time.

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Ethical approval

Studies were performed in accordance with IRB requirements at Albany Medical Center.

CRediT authorship contribution statement

Kathleen A. Stellrecht: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization. **Jesse L. Cimino:** Data curation, Formal analysis, Investigation. **Lisa I. Wilson:** Data curation, Investigation. **Vincente P. Maceira:** Investigation. **Shafiq A. Butt:** Project administration, Resources, Supervision.

Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.104204.

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