August 02, 2020

Vineeta Bajaj, Ph.D.
Review Editor
JoVE

Re: MS#: JoVE61670R1 "Evaluation of Capnography Sampling Line Compatibility and Accuracy When Used with a Portable Capnography Monitor"

Dear Dr. Bajaj,

We would like to thank the editors and reviewers for their valuable comments on our manuscript. We herein submit a revised version of the manuscript addressing the reviewer comments, with our point-by-point responses below.

Best regards,
Ruben D. Restrepo

EDITORIAL COMMENTS

Editorial Comment 1: Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

Author Response: Thank you for this suggestion. We have carefully reviewed the manuscript to ensure there are no spelling or grammatical errors.

Editorial Comment 2: Protocol Language: Please ensure that ALL text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.
1) Examples NOT in the imperative : 2.6.1

Author Response: We thank the editors for this comment, and have revised the protocol language to ensure all steps are written in the imperative tense, as highlighted below:

> 2.6.1 Calculate the maximum respiratory rate using the measured rise time for the sampling line and a 1:1 breath ratio, using the following equation: ~~For a 1:1 breath ratio,~~

~~the maximum respiratory rate represents the fastest allowed respiratory rate without impacting $ETCO_2$ accuracy when the time required for inhalation and exhalation are the same. This can be calculated using the measured rise time for the sampling line:~~

$$Maximum\ Respiratory\ Rate\ (BPM) = 30\ \text{sec} \div Rise\ time\ for\ sampling\ line\ (\text{sec})$$

where 30 sec represents the cumulative time used to exhale during 1 min (1:1 inhalation:exhalation time).

Note: For a 1:1 breath ratio, the maximum respiratory rate represents the fastest allowed respiratory rate without impacting $ETCO_2$ accuracy when the time required for inhalation and exhalation are the same.

2.6.2 Calculate the maximum respiratory rate using the measured rise time for the sampling line and a 1:2 breath ratio, using the following equation: ~~For a 1:2 breath ratio, the maximum respiratory rate represents the fasted allowed respiratory rate without impacting $ETCO_2$ accuracy when the time used to exhale is twice as long as the time used to inhale. This can be calculated using the measured rise time for the sampling line:~~

$$Maximum\ Respiratory\ Rate\ (BPM) = 40\ \text{sec} \div Rise\ time\ for\ sampling\ line\ (\text{sec})$$

where 40 sec represents the cumulative time used to exhale during 1 min (1:2 inhalation:exhalation time).

Note: For a 1:2 breath ratio, the maximum respiratory rate represents the fastest allowed respiratory rate without impacting $ETCO_2$ accuracy when the time used to exhale is twice as long as the time used to inhale.

In addition, we revised the protocol headings to ensure use of the proper voice:

1. **~~Measurement of~~ Measure sampling line tensile strength**
2. **Measure rise time ~~test~~ and sampling line accuracy**
3. **~~Measurement of~~ Measure $ETCO_2$ accuracy as a function of respiratory rate**
4. **~~Measurement of~~ Measure $ETCO_2$ accuracy in the presence of supplemental O_2**

Editorial Comment 3: Protocol Detail: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please ensure that all specific details (e.g. button clicks for software actions, numerical values for settings, etc) have been added to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:
1) 3.3.2: Mention software steps.
2) 3.3.: how?

Author Response: As requested, we have added specific details, including button clicks and numerical values for settings, to the protocol steps as outlined below:

2.1.2.5 Open the ~~LabVIEW~~ jig software and define the test parameters as follows: Air:

CO_2 ratio 1:1; Air time = 3 seconds, CO_2 time = 3 seconds, 10 cycles, rise time measurement length: none.

2.1.5 Compare the rise time value to the margins and confirm it is inside the specification limits, pre-defined as rise time background < 60 msec and rise time of a control sample, a 15 cm PVC tube, 0.95 mm internal diameter, equal to 39 ± 5 msec.

2.1.6 Compare the delivery time to the margins and confirm it is inside the specification limits, predefined as background delivery time <100 msec and delivery time of a control sample, a 15 cm PVC tube, 0.95 mm internal diameter, equal to 152 ± 5 msec.

3.3.2 ~~Using~~ Open the ~~control~~ breath simulator jig software, and ~~calibrate~~ set the ~~a~~ duty cycle to 50%.

3.3.3 Test for leaks in the system using a leak testing jig.

3.3.3.1 Connect the sampling line to the CO_2 port on the leak testing jig.

3.3.3.2 Create a kink in the sampling line to prevent CO_2 from exiting the end of the sampling line.

3.3.3.3 Using a flow rate of 50 mL/min CO_2, allow the pressure in the sampling line to increase to 300 mmHg, then stop adding CO_2.

3.3.3.4 Observe if the pressure in the sampling line remains the same or decreases. If the pressure decreases, this confirms a leak in the system, and a new sampling line should be applied in Step 3.2.

Editorial Comment 4: Protocol Highlight: Please ensure that the highlightin is under 2.75 pages (including line spaces).

Author Response: Thank you for this comment. We have removed some of the protocol highlighting to ensure that it is under 2.75 pages total.

Editorial Comment 5: Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Author Response: We reviewed the discussion section to ensure that it addresses the 5 discussion topics noted above. Key portions of the discussion section, including text added to

address these topics, are as follows:

1) Modifications and troubleshooting:

To apply the rise time findings to a clinical setting, we performed two tests to examine $ETCO_2$ accuracy when sampling lines were connected to a portable capnography monitor via a manikin. For both tests, we needed to modify the default capnography monitor settings to allow the monitor to recognize cross-paired sampling lines.

We needed to modify the rise time measurement parameters to remove the upper time limit on the rise time jig, so that the rise time could be collected for all sampling lines before the measurement period ended. The long rise time observed for some capnography sampling lines could reflect an increased volume of dead space in these sampling lines.

2) Limitations of the technique:

One limitation of this testing system is the continuous, gradual increase in force applied to the sampling line, as opposed to a sudden strong force, which could be encountered in clinical settings.

Although a rapid breathing rate of 150 BPM is unlikely to be encountered clinically, we determined the accuracy of each sampling device at this breath rate because it is considered the technical upper limit for many capnography sampling lines. While a respiratory rate of 150 BPM is non-physiologic, our bench test highlights that while some capnography sampling lines were accurate across the full technical range of respiratory rates, other sampling lines failed to achieve the same accuracy standard.

The main limitation of the $ETCO_2$ tests is that the tests are performed using a manikin and a controlled breathing system, as opposed to a human subject, in which breathing patterns vary between individuals.

3) Significance with respect to existing methods:

Although tensile strength tests are often performed on other types of medical devices, our method was unique in that it examined the tensile strength of each segment of the capnography sampling line. Therefore, in addition to determining ~~measuring~~ the tensile strength of each sampling line component, it also allowed for identification of the overall weak point of the complete sampling line.

…we determined the maximum respiratory rate and exhalation time for two unique breathing patterns, defined by inhalation:exhalation ratios equal to 1:1 and 1:2. This unique aspect of our analysis allowed us to evaluate the accuracy of measured CO_2 in circumstances that represent patients whose breathing pattern is uniform or whose exhalation time lasts longer than their inhalation time.

…similar to a previous study, we controlled respiratory rate using a respiratory rate controller, and monitored the resulting $ETCO_2$ measurements for each sampling line.

Importantly, many of the sampling lines failed to maintain $ETCO_2$ accuracy upon an increase in respiratory rate or upon the introduction of supplemental O_2, which is consistent with previous assessments of capnography accuracy. Together, our findings are consistent with previous bench tests that successfully measure the accuracy of capnography sampling lines.

4) Future applications:

As a validated instrument, the jig used to measure the tensile strength of the capnography sampling lines could be used for other applications, such as measuring the tensile strength of other sampling tubes and medical devices that have the potential to experience tension in a clinical setting.

Given that many of the sampling lines cross-paired to the capnography monitor exhibited reduced $ETCO_2$ accuracy in clinically relevant circumstances, care should be taken to ensure that any cross-paired commercial sampling lines and monitors are validated before being used to monitor patient ventilation status.

5) Critical steps within the protocol:

Although tensile strength tests are often performed on other types of medical devices, our method was unique in that it examined the tensile strength of each segment of the capnography sampling line. Therefore, in addition to determining ~~measuring~~ the tensile strength of each sampling line component, it also allowed for identification of the overall weak point of the complete sampling line.

…we determined the maximum respiratory rate and exhalation time for two unique breathing patterns, defined by inhalation:exhalation ratios equal to 1:1 and 1:2. This unique aspect of our analysis allowed us to evaluate the accuracy of measured CO_2 in circumstances that represent patients whose breathing pattern is uniform or whose exhalation time lasts longer than their inhalation time.

A key component of this test was the use of a pre-defined set of respiratory rates ranging from 10 to 150 BPM, to determine $ETCO_2$ accuracy across respiratory patterns that patients could exhibit.

Similar to the $ETCO_2$ accuracy test with varying respiratory rate, in this test, a key step in the protocol was to measure $ETCO_2$ accuracy across multiple supplemental oxygen flow rates.

Editorial Comment 6: Figure/Table Legends: Include a reference for Suppl File 1.

Author Response: Thank you for this comment. As requested by Reviewer 1, we have moved Supplementary Figure 1 to the main manuscript, and as such, have updated the Figure and Table Legends to reflect this. In the revised manuscript, Figure 4 (formerly Supplementary Figure 1A-B) is referenced in the results section entitled '*$ETCO_2$ accuracy as a function of respiratory rate*' and Figure 6 (formerly Supplementary Figure 1C-D) is referenced in the results section entitled '*$ETCO_2$ accuracy in the presence of supplemental oxygen*'.

Editorial Comment 7: References: Please spell out journal names.

Author Response: We have updated the references so that the full journal names are listed for each entry.

Editorial Comment 8: Commercial Language: JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Nafion, compact RIO, LabVIEW, SAS (SAS Institute Inc, Medtronic, Respironics and Medtronic, (Respironics , Flexicare, Salter Labs, Hudson, Westmed, Ventlab,
1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.
2) Please remove the registered trademark symbols TM/R from the table of reagents/materials.
3) Since you are comparing various systems, we suggesting labeling them system 1, 2, 3 etc and defining the labels in the table of materials.
4) Remove all product names from all figure and tables.

Author Response: Thank you for this important comment. We have removed the following

commercial language from the manuscript:

Nafion replaced with *humidifier* in all manuscript text, tables, and figures
Compact RIO replaced with *jig controller and power supply* in the manuscript text
LabVIEW replaced with *jig software* in the manuscript text
SAS replaced with *statistical software* in the manuscript text

In addition to the specific terms outlined above, we removed all commercial names for the capnography sampling lines from the manuscript text, tables, and figures, and as suggested, created a system to label the capnography sampling lines using numbers 1 through 16. These are defined by the manufacturer and product name in the Table of Materials, and the product names and manufacturers, including *Respironics, Flexicare, Salter Labs, Hudson, Westmed, Ventlab, and Medtronic*, are no longer used in the manuscript text, tables, and figures.

Finally, we have verified that registered trademark symbols are not included in the Table of Materials.

Editorial Comment 9: If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Author Response: Our figures and tables are original and not previously published.

REVIEWER 1

This is well written manuscript and performed a good comparisons among several sampling tube. They considered the tensile strength, rise time, $ETCO_2$ accuracy as a function of respiratory rate, and $ETCO_2$ accuracy in the presence of supplemental O_2 as assessment parameters for several sampling tube. The method is well written and explained. The finding of this research is analysed used well established method namely, Bland Altman plot.

Reviewer 1, Comment 1: Line 509; Together, these tests suggest that devices with a longer rise time have a lower maximum accurate respiration rate and exhibit low $ETCO_2$ accuracy at the maximum accurate respiration rate.

Do you want to say that the bad performance of other sampling tube rather than medtronic, happened due to longer rise time, hence it depends upon the CO_2 sensor response time, having lower rise time, may provide similar results.

Author Response: We thank the reviewer for this question regarding the relationship between rise time and sensor response time. Importantly, the CO_2 sensor response time equals the total of (1) the rise time of the sampling line and (2) the delay time, defined as the time required for the monitor to calculate $ETCO_2$. Since the same capnography monitor was used for all bench tests, the delay time is equal across all 16 capnography sampling lines reported. Therefore, the performance of the sampling lines does not require consideration of the CO_2 sensor response time (a constant value), but instead can be compared by the rise time alone.

Reviewer 1, Comment 2: As, relatively high sampling rate (i.e. 150 ml-min-l), may reduce the response time which indirectly reflect on rise time, may provide a better way to compare the sampling tube, I mean, the sampling tube which does perform well on higher sampling rate, may have similar results. The experiment should be performed with higher sampling flow rate to see the impact of rise time.

Author Response: We appreciate the reviewer's suggestion to perform the experiment with a higher sampling flow rate. Importantly, the rise time experiment was performed with the capnography monitor set at its maximum sampling flow rate, which is 50 mL/min. Therefore, we did not perform the experiment again with a higher sampling flow rate than originally used. However, we did add this important detail to the Protocol section as follows:

> *2.1.2.4 Calibrate the air and CO_2 flow to 10 L/min and the gas sampling rate to 50 mL/min using a mass flow meter and a dedicated restrictor. Note: The maximum sampling rate of the capnography monitor is 50 mL/min.*

Reviewer 1, Comment 3: I am curious to see the result of Bland altman plot with 95% confidence interval with upper and lower limit from its mean value, rather than bar graph.

Author Response: Thank you for this comment. Our initial manuscript submission included a supplementary figure of Bland Altman plots, displaying the bias with 95% confidence intervals for both $ETCO_2$ bench tests. Importantly, our revised manuscript includes these plots as Figures 4 and 6, for $ETCO_2$ accuracy as a function of respiratory rate and $ETCO_2$ accuracy in the presence of supplemental O_2, respectively. We hope that moving these plots to the main manuscript will avoid them potentially being missed by readers. The figure legends are now as follows:

> ***Figure 4: Bland-Altman plot for $ETCO_2$ measures by (A)** Matched sampling lines as a function of increasing respiratory rate and **(B)** Cross-paired sampling lines as a function of increasing respiratory rate.*

Figure 6: Bland-Altman plot for $ETCO_2$ measures by (A) *Matched sampling lines as a function of increasing supplemental O_2 flow rate; (**B**) Cross-paired sampling lines as a function of increasing supplemental O_2 flow rate.*

Reviewer 1, Comment 4: The sampling tube utilized from Medtronic are five that is comparatively more than the other company, which may provide bias results.

Author Response: We thank the reviewer for this important comment. As explained in the revised manuscript, we compared the bench test results between sampling lines from the same manufacturer as the capnography monitor (Medtronic, labeled in the manuscript as 'matched' sampling lines) vs sampling lines from alternate manufacturers (labeled in the manuscript as 'cross-paired' sampling lines). Since the key comparison was the performance of matched vs cross-paired sampling lines, we felt it necessary to include more than 1 matched sampling line across the bench tests, so that the results are not biased by a single matched sampling line. In addition, since the capnography sampling line designs vary by manufacturer, including multiple styles of matched Medtronic sampling lines allows for a more equal comparison against the varied designs tested from other manufacturers. In this way, we ensured an 'apples to apples' comparison, as opposed to an 'apples to oranges' comparison among the designs of the matched and cross-paired sampling lines. For these reasons, we have opted to retain the comparisons as in the originally submitted manuscript, and we added the following text at the beginning of the Protocol to clarify the comparison between matched and cross-paired sampling lines:

The capnography sampling lines used in these bench tests included 16 adult, pediatric, and neonatal capnography sampling lines from 7 commercial sources. Among the 16 sampling lines included in the bench tests, 5 sampling lines were from the same manufacturer as the capnography monitor utilized for the bench tests ('matched'), and 11 sampling lines were from alternate manufacturers ('cross-paired') (Table of Materials).

Reviewer 1, Comment 5: The balnd altman analaysis should provide the in main text rather than in supplementary.

Author Response: As suggested by the reviewer, we have moved the Bland Altman plots to the main manuscript, where they are reported in the revised manuscript as Figures 4 and 6. The figure legends are now as follows:

Figure 4: Bland-Altman plot for $ETCO_2$ measures by (A) *Matched sampling lines as a function of increasing respiratory rate and **(B)** Cross-paired sampling lines as a function of increasing respiratory rate.*

Figure 6: Bland-Altman plot for $ETCO_2$ measures by (A) *Matched sampling lines as a function of increasing supplemental O_2 flow rate; (**B**) Cross-paired sampling lines as a function of increasing supplemental O_2 flow rate.*

Reviewer 1, Comment 6: What is reference rise time (60 msec) ? if I am not wrong line (205) Record the background rise time and ensure the result is less than 60 msec. There are the sensor having lower response time comet, Sprint IR.

Author Response: The reviewer is correct that the background rise time, measured during calibration, is <60 msec. In addition, the rise time using a control sample, defined as a 15 cm PVC tube, 0.95 mm internal diameter, is expected to be 39 ± 5msec. Although not previously defined in the manuscript, the acceptable background delivery time is defined as <100 msec, with delivery time of a control sample (15cm PVC tube, 0.95 mm internal diameter) equal to 152 ± 5 msec. We have added these details to the Protocol section of the manuscript as below:

> *2.1.5 Compare the rise time value to the margins and confirm it is inside the specification limits, pre-defined as rise time background < 60 msec and rise time of a control sample, a 15 cm PVC tube, 0.95 mm internal diameter, equal to 39 ± 5 msec.*
>
> *2.1.6 Compare the delivery time to the margins and confirm it is inside the specification limits, predefined as background delivery time <100 msec and delivery time of a control sample, a 15 cm PVC tube, 0.95 mm internal diameter, equal to 152 ± 5 msec.*

Importantly, while other capnography monitors, such as the Sprint IR sensor mentioned by the reviewer may have a different response time, the purpose of our bench tests was to compare capnography sampling lines to one another using a single type of capnography monitor. Exploration of performance differences between multiple capnography monitors was out of the scope of this analysis, but is certainly a valuable topic for future studies.

REVIEWER 2

Reviewer 2, Comment 1: There are two different technologies for clinical capnography and two very different uses of clinical capnography. Types of capnography include in-line version and the side-stream version, capnography for breathing circuits in intubated patients and capnography for non-intubated patients using a nasal cannula. To bring clarity to this while concurrently orienting the reader to this being a study specifically on "nasal cannula capnography" would really help those who heavily use capnography hone in on what this particular study is focusing on while at the same time educating those who aren't as experienced on the diversity of types and uses of capnography. I do note that the last sentence of the introduction describes capnography as being used in intubated patients, but it does not bring clarity to the fact that intubated patients can use in-line or side-stream and that this is a

differently designed sample line.

Author Response: This is an excellent comment. As the reviewer notes, we initially provided little background on mainstream vs sidestream capnography, and have revised the introduction to include clarification that this study was focused on sidestream capnography, to compare performance of nasal cannula sampling lines. Key modifications to the Introduction section are as follows:

> *Inherent in the use of capnography is reliance on a device that provides the clinician with an accurate assessment of a patient's ventilatory status. Capnography monitoring can be either sidestream, in which exhaled breath is diverted to a monitor by a nasal cannula and tubing, or mainstream, in which exhaled breath is measured at the source without diverting the sample. Mainstream capnography is most often used in intubated patients, whereas sidestream capnography is used for both intubated and non-intubated patients. One important component of sidestream capnography is the sampling line…*
>
> *For example, nasal cannula sampling lines can have up to 10 connections between the nasal cannula, humidifier, $ETCO_2$ sampling line, and O_2 delivery tubes (Figure 1).*
>
> *The performance of nasal cannula sampling lines can be evaluated by a variety of tests such as the overall weak point and rise time.*
>
> *Another critical element of sidestream capnography monitoring affected by sampling line design is rise time…*
>
> *The purpose of this study was to determine the compatibility and accuracy of commercially available sidestream capnography sampling lines used in conjunction with a portable capnography monitor.*

Reviewer 2, Comment 2: As the text of the article develops it seems to evolve into a comparison of Medtronic sample lines vs non-Medtronic sample lines. Considering that the authors all have Medtronic connections it would be in order to clarify "the reason" for separating out the Medtronic lines. Ironically it would both allow more description regarding the superior performance of the Medtronic lines while also giving a reason for the Medtronic vs non-Medtronic grouping, which currently has the appearance of being associated with corporate bias. I think that this issue can be clarified because there does seem to be a qualitative difference between the Medtronic and non-Medtronic sample lines. If there is no result related basis for grouping Medtronic vs non-Medtronic then it would be better not describe this group comparison. This is a major issue with the paper that should be addressed.

Author Response: We thank the reviewer for highlighting this topic, which was also raised by Reviewer 1. As explained in the revised manuscript, we compared the bench test results between sampling lines from the same manufacturer as the capnography monitor (Medtronic, labeled in the manuscript as 'matched' sampling lines) vs sampling lines from alternate manufacturers (labeled in the manuscript as 'cross-paired' sampling lines). By re-defining these groups of sampling lines into matched vs cross-paired groups, as opposed to Medtronic vs non-Medtronic, the emphasis of our observations is on the qualitative difference between these groups of sampling lines, and less on the manufacturer, avoiding potential corporate bias. In addition, we consider the matched Medtronic sampling lines to be an appropriate control group for comparison against the cross-paired sampling lines' performance with a Medtronic capnography monitor, and as mentioned in our response to Reviewer 1, Comment 4, we included multiple styles of matched sampling lines to allow for a more equal comparison against the varied sampling line designs tested from other manufacturers.

Among many manuscript modifications to remove references to specific sampling line manufacturers, we added the following text at the beginning of the Protocol to clarify the comparison between matched and cross-paired sampling lines:

> *The capnography sampling lines used in these bench tests included 16 adult, pediatric, and neonatal capnography sampling lines from 7 commercial sources. Among the 16 sampling lines included in the bench tests, 5 sampling lines were from the same manufacturer as the capnography monitor utilized for the bench tests ('matched'), and 11 sampling lines were from alternate manufacturers ('cross-paired') (Table of Materials).*

Reviewer 2, Comment 3: Why were the various respiratory rate points chosen? Why is it important or significant that a sample line performs at a respiratory rate of 150 breaths per minute? As a clinician I can't imagine a person, even a neonate breathing at 150 BPM and just need to understand why performance at this level significant or why this rate was chosen. It would be important to mention that the high respiratory rates are non-physiologic, but that some of the lines performed so well that they could function with these non-physiologic rates.

Author Response: This is an excellent question. We chose a variety of respiratory rates to reflect possible breathing scenarios encountered in clinical settings. Importantly, we included up to 150 BPM because this respiratory rate is defined as the technical upper limit for many capnography sampling lines. Thus, even if a respiratory rate of 150 BPM is unlikely to occur in a clinical setting, as a bench test, we felt it was appropriate to test the full technical range of the devices. We have added this detail to the Discussion section as reflected below:

> *Although a rapid breathing rate of 150 BPM is unlikely to be encountered clinically, we determined the accuracy of each sampling device at this high breath rate because it is considered the technical upper limit for many capnography sampling lines. While a*

respiratory rate of 150 BPM is non-physiologic, our bench test highlights that while some capnography sampling lines were accurate across the full technical range of respiratory rates, other sampling lines failed to achieve the same accuracy standard.

Reviewer 2, Comment 4: Figure 3A is without proper captioning. The reader has to go back to the text of the article to understand that all of the lines were tested with a CO_2 level of 34mmHg. What is not clear and not addressed is why the sample lines were reading higher and much higher CO_2 levels than 34 mmHg per this graph. The issue of captioning applies to all of the figures.

Author Response: Based on the reviewer's comment, we have added the following detail to the figure legend for Figure 3:

Figure 3: $ETCO_2$ accuracy of adult and pediatric capnography sampling lines as a function of respiration rate. Measured $ETCO_2$ values for (A) Adult and (B) Pediatric and Neonatal capnography sampling lines across a range of respiratory rates from 10 to 150 BPM. In all cases, the expected $ETCO_2$ value is 34 mmHg.

Please note that the expected $ETCO_2$ value of 34 mmHg was already highlighted in the Figure 5 legend:

Figure 5: $ETCO_2$ accuracy of capnography sampling lines in the presence of increasing supplemental oxygen. $ETCO_2$ accuracy is reported for (A) No supplemental oxygen; (B) 2 L/min supplemental oxygen; (C) 4 L/min supplemental oxygen; and (D) 6 L/min supplemental oxygen. The green line at 34 mmHg represents the expected $ETCO_2$ value across all measurements.

With respect to the sampling lines that read $ETCO_2$ levels higher than 34 mmHg at higher respiration rates, this is partially addressed in the Results section, in which we pre-defined the acceptable accuracy for readings between 0-38 mmHg and readings >38 mmHg:

The expected $ETCO_2$ in the presence of 5% CO_2 was 34 mmHg at ambient pressure, and the range predefined as acceptable accuracy was ±2 mmHg for readings between 0-38 mmHg and ±5% of the reading + 0.08 for every 1 mmHg above 38 mmHg.

The high $ETCO_2$ readings are likely due to the sampling line design and the amount of dead space present within the sampling lines, where less dead space may result in increased $ETCO_2$ measurements, and more dead space within the sampling line leads to mixed breath samples (Figure 2A). In the case of a large volume of dead space, the amplitude of the mixed breath becomes so low that the capnography monitor does not recognize it as a valid breath (Figure 2B). While this concept was addressed in the Discussion section, we have added more detail to address the topic:

This accuracy could be due to the design of the sampling lines, such that those with higher friction or larger dead space volume result in lower resolution breath samples at increased respiratory rate, similar to what we observed in the rise time test. While the sampling lines with high $ETCO_2$ readings may contain less dead space that enable them to deliver discrete breath samples, the error of $ETCO_2$ readings above 38 mmHg was pre-defined as ±5% of the reading + 0.08 for every 1 mmHg above 38 mmHg. This could partially explain why the $ETCO_2$ readings were increased above 34 mmHg during high respiratory rate in some sampling lines. In contrast, the sampling lines with low or zero $ETCO_2$ readings may contain more dead space, resulting in mixed breath samples that the capnography monitor does not recognize as valid breaths, and thus reports as no breath.

Reviewer 2, Comment 5: I as a reader have a perception that the "breathing jig" design may be important. Unfortunately I don't understand how this jig is set up, how the breaths were simulated and whether its design may have impacted sample line performance. Considering the fact that some of the sample lines had unbelievably poor performance (0 breaths detected when oxygen was flowing), why people would be purchasing a useless sample line, unless the clinical performance on actual humans was different. Thus I wonder about the realism of the "breathing jig".

Author Response: We thank the reviewer for this question. With respect to the breathing jig, we have added a more detailed description of the jig to the Protocol section of the manuscript:

3.3 Prepare and calibrate the breath simulator jig, to control the simulated respiratory rate. Note: The breath simulator jig is composed of a 2-way electrical operating valve, allowing for precise control of the flow of CO_2 and N_2 to the manikin, to simulate human breathing.

Although we do not speculate in the manuscript on the reason why poor-accuracy sampling lines are purchased, it is important to highlight potential reasons for the poor accuracy observed in our bench tests. The breathing jig is simply used to control the amount of CO_2 'exhaled' by the manikin, to represent a human breath. While this bench test may not perfectly reflect the real breath patterns of humans, it is designed as a controlled system to mimic breathing. We highlighted this as a limitation in the Discussion section:

The main limitation of the $ETCO_2$ tests is that the tests are performed using a manikin and a controlled breathing system, as opposed to a human subject, in which breathing patterns vary between individuals.

Our interpretation of the poor performance of some capnography sampling lines in the presence of supplemental O_2 is that this is not an experimental artifact, but rather, a reflection of the capnography sampling line design. In particular, most of the dead space within a sampling line is within the cannula, which delivers oxygen to the patient and collects exhaled

breath for $ETCO_2$ measurement. If the sampling line is designed with a large dead space in the cannula, during oxygen delivery, the O_2 can become mixed with the exhaled breath, resulting in dilution of the exhaled breath and a low amplitude $ETCO_2$ curve that the capnography monitor does not detect as a valid breath. Therefore, if the sampling line, and in particular, the nasal cannula, is not carefully designed, in the presence of higher flow oxygen, $ETCO_2$ cannot be accurately measured by the capnography sampling line and monitor. We have added this to the discussion as below:

> *In particular, similar to our observations upon increase of the respiratory rate, the $ETCO_2$ readings for sampling lines 2 and 5 dropped to 0 mmHg in the presence of supplemental O_2, suggesting that their $ETCO_2$ accuracy when cross-paired with a capnography monitor is very low. This may be due to the design of the sampling lines, and in particular, the nasal cannula design, which is designed to both deliver oxygen to a patient and collect breath samples from a patient. If the nasal cannula contains a large amount of dead space, mixing of the supplemental oxygen and the exhaled breath can occur, resulting in low amplitude, mixed breaths that the capnography monitor does not detect as exhaled breath. In such a case, the $ETCO_2$ measurement would drop to zero, as we observed with some of the cross-paired sampling lines tested.*

We thank the reviewers for the time and effort they have devoted to critically reviewing our manuscript. The reviewer's comments are much appreciated and we hope the changes made in response to their recommendations have strengthened the manuscript for publication in *JoVE*.

Please feel free to contact me with any questions.

Kind regards,

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