

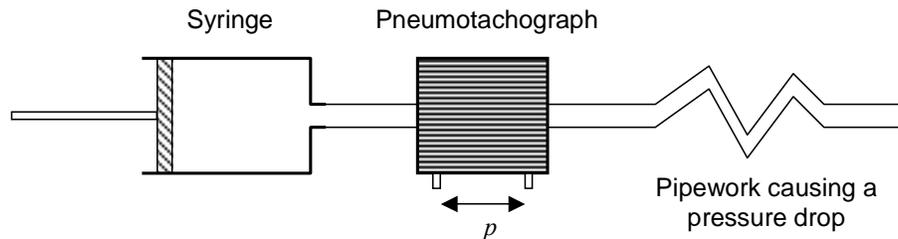
Pneumotachometer calibration

Overview A pneumotachometer or pneumotachograph (PT) is a type of gas flow meter that is widely used for respiratory monitoring in physiological research and medicine. It is a differential pressure transducer with an output approximately proportional to flow. However, PT output varies somewhat depending on factors such as temperature, humidity and the nature of the equipment to which it is connected. Thus it must be recalibrated regularly under conditions as close as possible to those under which physiological measurements are performed.

It is tricky to generate a series of known constant flow rates that would be needed for a conventional calibration procedure. Therefore, the Spike2 script described here generates a PT calibration curve based on a recording of PT output in response to a sequence of inward and outward flows at various rates generated using a calibrated syringe. It is possible to derive a calibration for the non-linear flow meter behaviour given that the final volume of a series of syringe strokes is always the same.

The maths of calibrating a pneumotachometer

The experimental situation is:



The syringe has a known volume and the system is calibrated by performing a series of syringe strokes, all of equal volume, alternately emptying and filling the syringe. The flow is measured by looking at the pressure difference across the Pneumotachograph (PT). The pressure difference is related to the flow rate through a transfer function that will depend on temperature, pressure and humidity. As we will assume that the calibration and use will be done at the same temperature and atmospheric pressure, and that the extra humidity on exhalation will be handled separately, we will assume that the true flow V' (dV/dt) can be written as a function of pressure difference p across the PT as:

$$V' = P(p)$$

where $P(p)$ is a polynomial in p ($a_0 + a_1p + a_2p^2 + a_3p^3 + \dots$) where the a_i are constants to be determined. However, we know that $P(0)$ is 0, so a_0 is 0 as a pressure difference of 0 corresponds to no flow. Sadly, things are not quite so simple as, if the pipe work causes a pressure difference in the PT from atmospheric P_A of P , then the formula becomes:

$$V' = P(p) * (P_A + P) / P_A$$

This correction $(1 + P/P_A)$ will be small in any reasonable system, as the pressure change should be small compared to P_A . If we make the reasonable assumption that the pressure drop P is proportional to flow rate, we can write this factor as $(1 + cV')$, where cV' is small compared to 1. So we can write:

$$\begin{aligned} V' &= P(p) * (1 + cV'), \text{ or} \\ V'(1 - cP(p)) &= P(p), \text{ or} \\ V' &= P(p) / (1 - cP(p)) \end{aligned}$$

Now $c P(p)$ is small compared to 1 and as $(1-x)^{-1}$ is approximately equal to $(1+x)$ if $x \ll 1$, we can write this to some approximation as:

$$V' = P(p) * (1+cP(p))$$
$$V' = P(p) + c P(p)^2$$

As the square of a polynomial is just another polynomial, we can write this as:

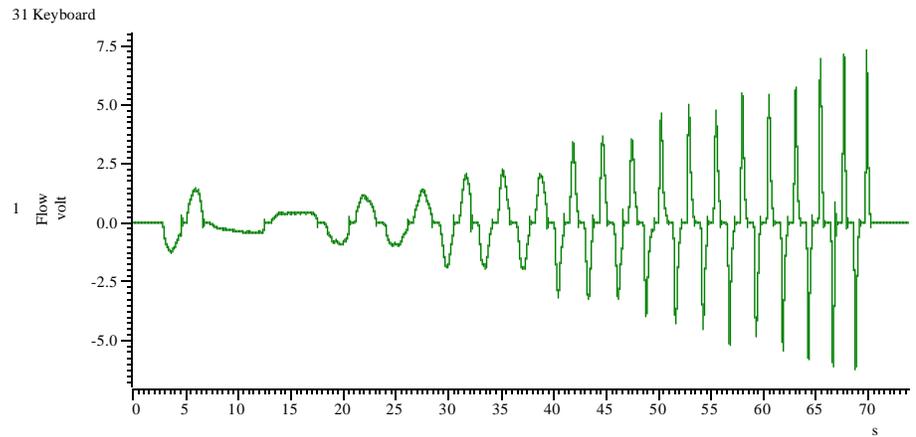
$$V' = Q(p)$$

Where $Q(p)$ is another polynomial in p , albeit of higher order. This also has the property that $Q(0)$ is 0.

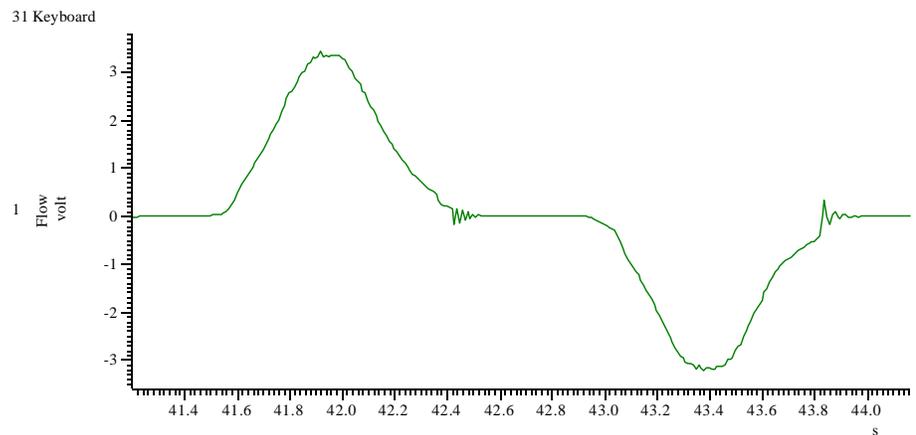
On the other hand, if the difference in pressure from atmospheric is not proportional to the flow but due to some external factor, then you must measure the pressure and apply a correction.

How to solve this

We propose to sample a series of syringe movements, each one filling or emptying the syringe, so each has a known volume. Here is a typical set of data for the calibration process:



and here is one cycle of syringe movement:



The point to note is that several cycles (19 in this case) of syringe movement have been done at different flow rates. The sampled signal is the pressure across the PT. We know that each cycle has moved the same volume of air through the PT (because at the start and end of each cycle, the pressure in the syringe is atmospheric as the flow through the system is 0).

For all the positive cycles, each of volume V we have for a cycle lasting from time 0 to t :

$$\int_0^t V' dt = \int_0^t Q(p) dt = V_s$$

where V_s is the volume of the syringe and V' is the derivative of volume with respect time (that is, flow) and $Q(p)$ is the function that converts the measured PT value into flow. When dealing with sampled data, each cycle is represented by N_c sampled points (N_1, N_2, N_3 for cycles 1..3) and the integrals become summations:

$$\hat{U} V' \hat{e} t = \hat{U} Q(p) \hat{e} t = V_s, \text{ sum is over the } N_c \text{ sampled points, } \hat{e} t \text{ is the sample interval.}$$

Put another way, for every cycle we have:

$$\hat{U} Q(p) = V_s / \hat{e} t$$

Now, $Q(p)$ is the polynomial $q_1 p + q_2 p^2 + q_3 p^3 \dots + q_n p^n$. There is no q_0 term as we know that there is no flow if there is no pressure difference. If you want to include q_0 as a check of the process, the matrix A and vector q and p (below) are expanded in an obvious way to include a $\hat{U} p^0$ term (that is $\hat{U} 1$).

We want to choose the q_i to get the best least-squares fit of our data, defined as the values that minimise the error over all the cycles. The error for a single cycle is:

$$E = (\hat{U} Q(p) - V_s / \hat{e} t)^2, \text{ which is the same as } (\hat{U}(q_1 p + q_2 p^2 + q_3 p^3 \dots + q_n p^n) - V_s / \hat{e} t)^2$$

summed over all the points in a cycle.

This is a standard minimisation problem and is solved by partially differentiating the expression with respect to each of the q_i in turn and setting the result to 0. The result for q_i for a single cycle is:

$$\begin{aligned} \hat{U} E / \hat{U} q_i &= 2(\hat{U}(q_1 p + q_2 p^2 + q_3 p^3 \dots + q_n p^n) - V_s / \hat{e} t) \hat{U} p^i = 0, \text{ or} \\ q_1 \hat{U} p \hat{U} p^i + q_2 \hat{U} p^2 \hat{U} p^i + q_3 \hat{U} p^3 \hat{U} p^i \dots + q_n \hat{U} p^n \hat{U} p^i &= (V_s / \hat{e} t) \hat{U} p^i \end{aligned}$$

The summation is over all cycles and over all points per cycle. If you want to fit an n^{th} order polynomial, there are n equations and n unknowns. This can be written as the matrix equation:

$$\mathbf{A} \mathbf{q} = \mathbf{b}$$

where for a third order polynomial, \mathbf{q} is:

$$(q_1, q_2, q_3)$$

\mathbf{b}^T is (the T means transpose as it is a column, not a row). In these expressions, the symbol \hat{U} means sum over all cycles, the \hat{U} symbol means sum within a cycle.

$$((V_s / \hat{e} t) \hat{U} \hat{U} p, (V_s / \hat{e} t) \hat{U} \hat{U} p^2, (V_s / \hat{e} t) \hat{U} \hat{U} p^3)$$

and \mathbf{A} is a 3 x 3 matrix:

$$\begin{array}{lll} \hat{U}(\hat{U}_p \hat{U}_p), & \hat{U}(\hat{U}_p^2 \hat{U}_p), & \hat{U}(\hat{U}_p^3 \hat{U}_p) \\ \hat{U}(\hat{U}_p \hat{U}_p^2), & \hat{U}(\hat{U}_p^2 \hat{U}_p^2), & \hat{U}(\hat{U}_p^3 \hat{U}_p^2) \\ \hat{U}(\hat{U}_p \hat{U}_p^3), & \hat{U}(\hat{U}_p^2 \hat{U}_p^3), & \hat{U}(\hat{U}_p^3 \hat{U}_p^3) \end{array}$$

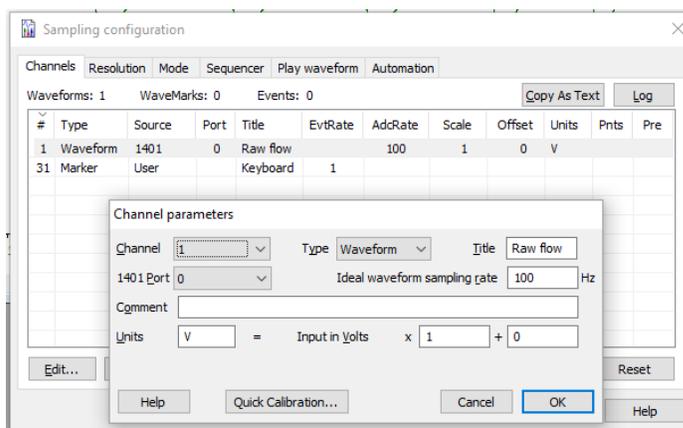
This can be solved for the q_i using standard matrix methods (for instance using the `MATsolve()` Spike2 script function).

Pneumotachometer calibration

Calibration procedure The script *PTCal_x.s2s* is a practical implementation of this calibration method. Keep it in the same folder as the other scripts in the *Resp* family, e.g., *scripts*, inside your *Spike_n* directory in *My Documents*.

Run it for the first time via the *Run script/Load and Run* option on the *Spike2 Script* menu. When the script toolbar appears, click on the *Quit* button. You should see that the script has installed a hotkey labelled *PTCal_x* on the *Spike2* script bar. In future, you can run the script by clicking on this button.

Recording syringe strokes In order to record a series of syringe strokes that form the basis of the calibration, you will need a sampling configuration containing one waveform channel with the title *Raw flow* sampled at 100 Hz calibrated in Volts with zero offset as shown below.



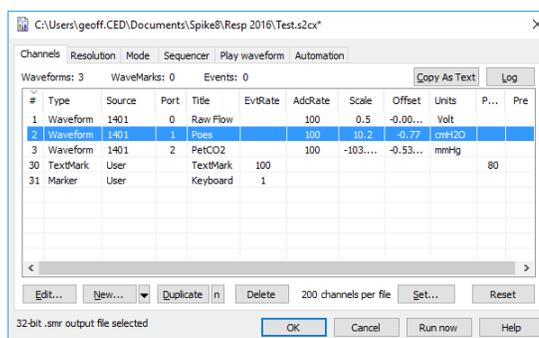
You can find a complete guide to creating a sampling configuration in the *Sampling Data* section of *Spike2 Online Help*. Save this configuration with a suitable title via *Save Configuration As...* on the *Spike2 File* menu and re-load it whenever you need to record a new calibration file.

Sample Now!



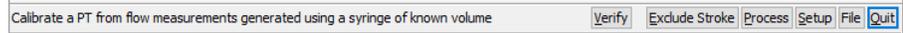
When ready to record, simply click on the *Sample Now!* icon on the *Spike2* toolbar followed by *Start* on the *Sampling Control* panel. (If the sampling controls do not appear then right-click on the application background (usually grey) and choose *Sampling Controls* from the context menu). Record the syringe strokes and save the file.

Prepare to calibrate Before running the calibration script, you need to have ready, the sampling configuration that you plan to use to make physiological recordings. The minimum requirement is a configuration similar to the one you used to record the syringe strokes. More likely, you will also wish to record other channels such as oesophageal pressure or CO₂ levels. Adding an online *TextMark* channel is also useful, since this allows you to add comments to the data file during recording. In *Spike2 v8*, the *Channels* page of a suitable sampling configuration would look something like this:



When you are satisfied with the configuration, save it to disk with a suitable name using the *Save configuration as...* option on the Spike2 File menu. The calibration curve of the flow meter will be built into the sampling configuration when you close the *PTCal* script.

Running the *PTCal* script The script generates a toolbar with 6 buttons:



Open a file Start by clicking on **F**ile and browse to a recording of the calibration procedure, that is, the PT response to successive inward and outward strokes of a calibrated syringe. A sampling rate of 100 Hz gives adequate resolution and a sequence of around 20 inward and outward strokes at a range of flow rates is usually sufficient to generate a good calibration curve. Ideally, there should be a delay of at least 1s between successive syringe strokes in order to reveal any standing offset at zero flow.

Set up Next, click on **S**etup and enter in the dialog: the volume of the syringe, the channel containing the PT signal and a channel to hold markers that indicate the start and end points of each syringe stroke. Select *New* to create a new marker channel and click on **O**K to continue. (Alternatively, you can select a pre-existing stroke marker to repeat a previous calibration.)

Drag **C**ursor (1) to a position just before the start of the first syringe stroke and **C**ursor (2) to a corresponding position after the end of the last stroke. Click **O**K on the toolbar (hotkey: *Enter*), to continue. A horizontal cursor will appear at the mid-point of the flow trace. If necessary, drag it *approximately* to the level that represents zero flow and click on **O**K or press *Enter*. At this point, the stroke marker toolbar appears.

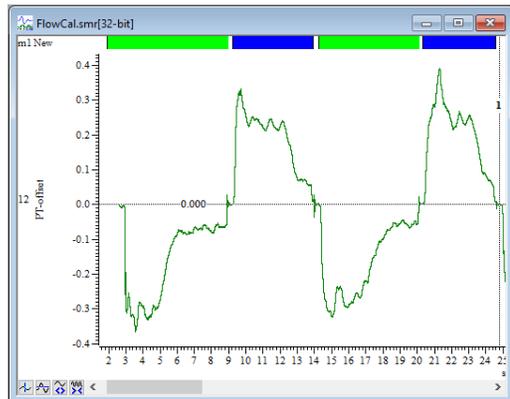
Stroke Marker toolbar



Two methods are available for marking the start and end of each syringe stroke.

Manual mode This method allows you to choose only the relevant parts of the trace and to exclude artifacts. Fetch **C**ursor (1) using the toolbar button and mark the onset of the first syringe stroke by dragging to a position of zero flow before the stroke onset. Click on **S**tart on the toolbar or press the *space bar* to add a marker. Next, drag the cursor to a point of zero flow at the end of the stroke and Click the **E**nd button or press the *space bar*. Repeat this procedure until the start and end of all of the syringe strokes have been marked. You can scroll to the next section of the trace by clicking on the **S**croll button (*hotkey: →*).

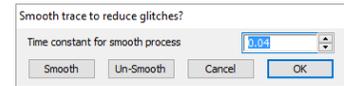
You should end up with a series of coloured bars marking the duration of inward (green) and outward (blue) strokes. If you make a mistake, you can use the **D**elete and **R**eset buttons to edit the markers. **D**elete removes a single syringe stroke marker (i.e. start *or* end) at or after the **C**ursor. **R**eset, deletes all of the markers so that you can start over. Press **D**one when you have finished marking all of the syringe cycles. The script will then check that the order of stroke markers makes sense. If there is a problem, you will have the option to reset the stroke marker



channel or to edit it. If you select **Edit** then the script will remove a suspect marker and let you inspect the trace and retry by pressing **Done** again.

Auto mode The **AutoDetect** button on the toolbar provides a quick (but possibly dirty!) method of marking the syringe strokes. The script measures the times at which flow crosses adjustable threshold levels set just above (and below) the baseline level and marks the end of one stroke and the start of the next at a point midway between these times. This method will place the markers mid-way between strokes and leaves virtually no gaps between syringe strokes. However, it *assumes* that the flow at these points is zero rather than measuring it. If the interval between syringe strokes contains significant noise, then it is advisable to mark the strokes manually so that noisy parts of the baseline can be excluded from the calibration procedure.

When you click on the **Auto** button, a dialog gives you the option to apply a smoothing process to the flow data. Smoothing should reduce the amplitude of glitches between the syringe strokes so that the threshold for reliable detection of stroke onset and end can be set lower. You can experiment, with different smoothing, time constants until you are satisfied with the results. Note, that any previous smoothing process is cleared automatically before a new one is applied.



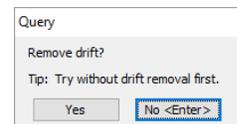
After smoothing, a dialog allows you to set the threshold for stroke detection. You can type a value into the selection box or click on the spinner arrows. Alternatively, you can drag the horizontal cursor in the flow channel to the required level. It is important to choose the lowest threshold consistent with avoiding artifacts triggering the stroke detector. Click on **OK** to automatically mark the syringe strokes.



The stroke marker will display and a dialog will show the number of strokes that were detected. If the number is correct, then you can click on **OK** to return to the main toolbar and proceed to the calibration procedure proper. If not, you can go back, re-adjust the stroke detection parameters and retry. Note that the smoothing process is removed automatically before the calibration is applied.

Exclude strokes This button allows you to exclude one or more syringe strokes from the calibration procedure. Simply, press the button, drag the cursor into the stroke you want to remove and click on the **Exclude** button on the toolbar. Click on the **Done** button to return to the main toolbar.

Process The calibration method requires that each syringe stroke starts and ends at zero flow. It will be seriously compromised if there is any drift in the level of the raw flow trace between the beginning and end of a stroke. The script gives you the option to remove such drift before calibrating via a dialog. If you choose drift removal the script does this by subtracting a straight line joining the start and end points of each stroke from the PT output before calibrating. If drift is indeed present then this procedure will improve the calibration. However, if there is no underlying drift then the method may be counter-productive. We suggest that you try both and establish which works best for you.

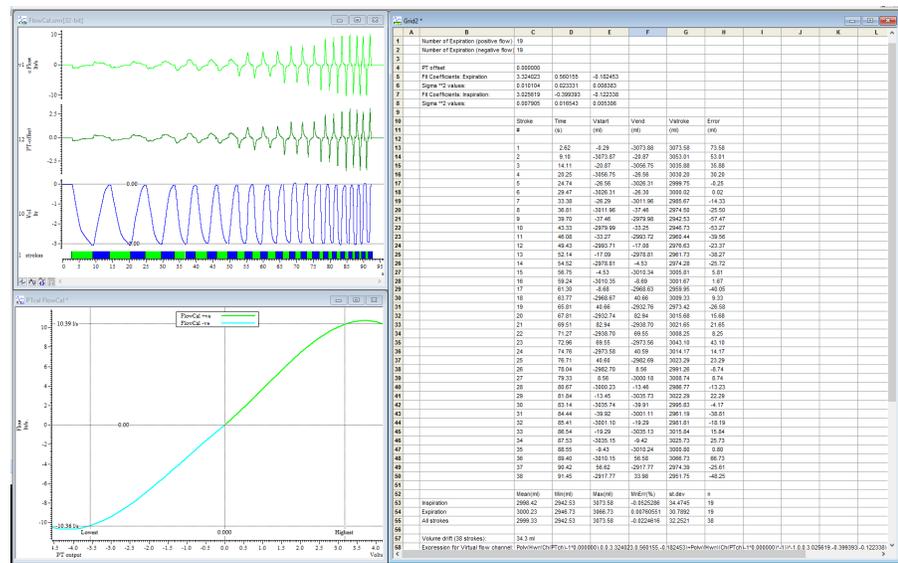


When you click on **Process**, the script performs the calculations outlined above for inward and outward flows separately. For each cycle, it builds the matrix **A** and the vector **b**. The results from each cycle are added into a master matrix and master vector for each flow direction. The script then solves the resulting matrix equations in order to generate, the q_i values (the coefficients) that convert the PT signal data into flow.

Thus, we derive two 3rd order polynomial expressions that represent the least squares best fit to the inward and outward flow data. The resulting calibration curves are calculated for PT outputs in the range -10V to +10V although only the most relevant range is displayed in the calibration curve. Cursors mark the lowest and highest flows found in the data file.

Typical results

A calibrated flow channel (*c.Flow*) is displayed in the original data file. This is a Virtual channel created by applying the polynomial correction to the PT signal. The data file also shows a volume channel (*Vol*) based on the calibrated flow with horizontal cursors marking the expected range based on the volume of the syringe.



The results table shows the offset that was removed from the raw flow signal prior to the fitting procedure, the coefficients of the best fit curves for inward and outward flow together with the corresponding σ^2 values.

There is also a table of start, end and stroke volumes for each syringe stroke together with the mean stroke volume and its standard deviation. Additional indications of the quality of the calibration are: the lowest and highest measured stroke volumes and the apparent drift in volume between the start and end of the calibration procedure. The final item is the expression required in a Virtual channel in order to generate a calibrated flow signal. You can use this to create a Virtual channel showing calibrated flow in a pre-existing data file by substituting the actual channel number of the PT signal into the expression in place of <PTchnr>.

The expression contains components for removing any standing offset from the raw flow signal so that zero Volts represents zero flow. Separate third order polynomial curve fits for inward and outward flows are then combined by means of half-wave rectification (*Hwr*). See the Spike2 Help on Virtual channels for further information on the elements of the flow calibration equation.

Save the calibration Press **Quit** to close the PT calibration script. During the closedown, you will have the option to save or discard the calibration curve and the results table via typical Windows *File Save As* dialogs.

Most importantly, you can incorporate the flow calibration into the sampling configuration that you use to record physiological data. This means that a calibrated flow channel based on the recorded PT signal will be generated online when that sampling configuration is used.

You will be prompted to switch the 1401 interface on in order to do this. The script then prompts you to browse to the relevant sampling configuration file and open it. A new data file based on this sampling configuration will display. Next, select the channel that records the PT signal in the accompanying dialog and press **OK**.

At this point any previous calibration will be deleted from the configuration, the new one is added and the data file closes. You can now save the updated configuration under a new name or the original one as you wish. The next time that you load this configuration and sample data, a virtual channel that converts PT output to calibrated flow will display online. You can use the *FV_Online.scx* script in conjunction with this sampling configuration in order to generate BTPS-corrected flow and volume traces online.

Verify This button will be enabled when you open a flow calibration data file that contains a raw flow channel and a channel of syringe stroke markers. In order to be recognised, both channels must be visible, the flow channel must have a title containing the string $\delta F/\delta t$ and the marker channel's title must include the string $\delta str/\delta t$. You can edit the channel titles to meet these criteria by double-clicking on the channel titles in the data file if necessary.

Clicking on this button allows you to apply an existing flow calibration to the current set of syringe strokes. You will be prompted to browse to and open a sampling configuration containing a flow calibration. When you click on **Open**, this calibration will be applied to the current syringe stroke data. The display will be similar to the one generated when you press **Process**. You can then decide whether the existing calibration is still valid, based on the calibration curve, the flow and volume traces added to the data file and the table of results.

If you press **Process** after **Verify**, then you can compare the existing calibration data with a new one in detail before deciding whether or not to update your sampling configuration.

Reference Tang Y, Turner MJ, Yem JS and Baker AB Calibration of Pneumotachographs using a calibrated syringe. *J Appl Physiol* **95**:571-576, 2003

At the time of writing this article was available via the link:
<http://jap.physiology.org/cgi/content/full/95/2/571>