Log that Clog: Automatically Detect and Manage Clogs on the iQue® High-Throughput Screening (HTS) by Cytometry Platform

Technical Note

Clogging is a widespread concern in cytometry, particularly when using sticky or aggregate-prone cells and sample types and can result in a loss of data. This document provides some information and practical tips about the clog detection and management feature available on the iQue® 5 HTS Cytometry Platform and within the iQue Forecyt® Software. This enables timely warnings when an issue arises and offers potential for sample protection through automated cancellation of the affected run.

Introduction

Flow cytometers are designed to interrogate the features of individual cells through the precise presentation of single cells in a fluid suspension passing through a flow cell. Due to this innate configuration of the instrument, the method is particularly susceptible to cellular and particulate aggregation, which can cause clogging of the flow cell and lead to catastrophic effects on data capture. Unlike other cytometers, the iQue® High-Throughput Screening (HTS) by Cytometry Platform uses an innovative sampling method to achieve its rapid sample acquisition, which is reliant on air gaps to separate samples. This document provides technical information on the clog detection and management feature present in the iQue® 5 HTS Platform, which is controlled within the iQue Forecyt® Software.

Recognizing a Clog

Using the iQue® 5 Platform, clogs can be automatically detected and warnings displayed, but it is still useful to understand how these clogs appear in the various data plots. Previous instrument versions do not have automated detection. Clogs are easily detected when looking at time data such as the well identification time histogram. As the iQue® technology uses accurate air gaps to separate samples, the length and frequency of both the air gap and sample can be tracked. Usually, flow cell clogs present as wider or smaller well collections before the gap of incoming events. In contrast, a clog in probe and tubing usually results in an abrupt stop in events. They usually present as an interruption of normal sampling sometimes followed by a burst of compressed wells as the clog breaks free (Figure 1). The other way to recognize a clog is to observe the sample slugs as they travel through the tubing. The slugs are small volumes of liquid that are separated by air gaps. If the slugs are not moving at all or are moving very erratically, you may have a partial or total clog.

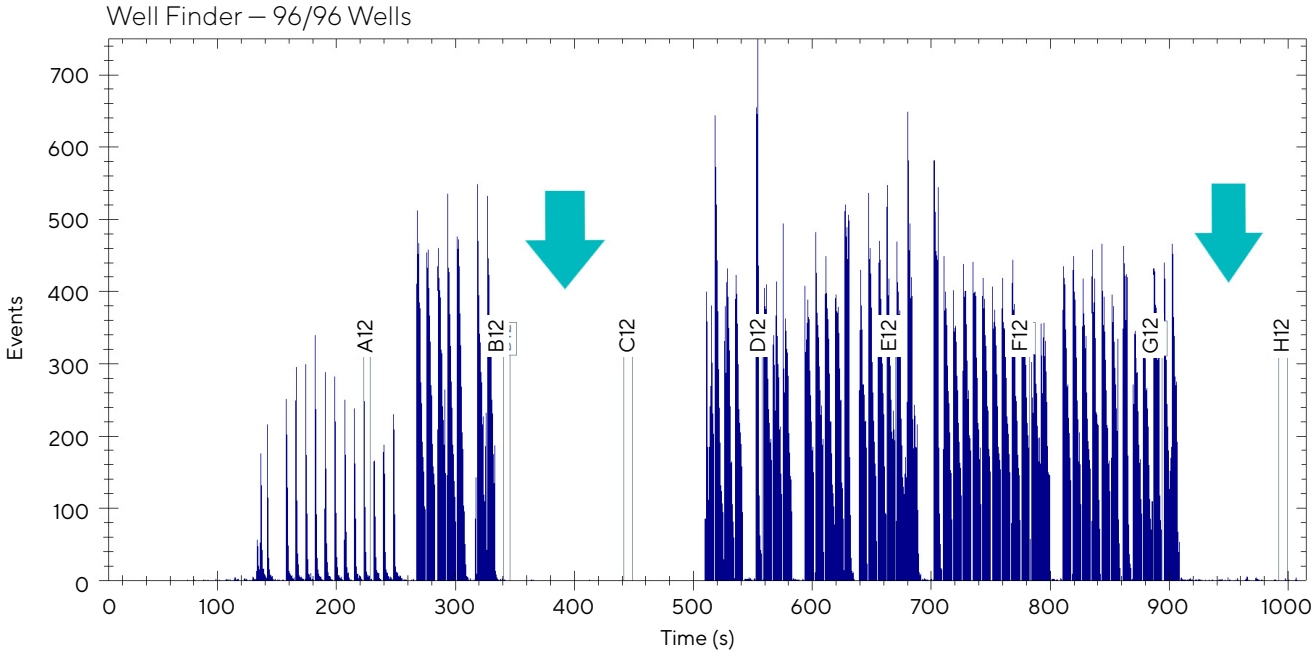


Figure 1: Example of Sample Clog. Sample data taken from a co-culture of human PBMC, Raji B cells and Jurkat T cells, sampled from a 96-well plate. Teal arrows signify where a clog occurred, shown as total loss of events.

iQue® 5 Bubble Sensor

Clogging is a pain point for many customers and, to address this concern, the iQue® 5 instrument has innovative design features to help address this. The overall design of the fluidics assembly has been improved compared to the previous iQue® 3 Platform. The material used for tubing has increased rigidity and resistance to pump pressures, which increases the operational flow. This prevents fluid path inconsistencies and reduces the frequency of clogs.

The iQue® 5 Platform is equipped with a bubble sensor on the front panel of the instrument, highlighted in the image below (Figure 2). This is a visual check for the air gap and displays as a green flashing light as the air gap passes through the sensor.

Under normal operation, the bubble sensor detects the air gap between the samples as they pass through the sample tubing, displaying a green light. This appears as a rhythmic flashing light, with the frequency dependent on the length of sip time. It provides the user with a visual indication of even air gaps. If a solid light or no light is visible, this indicates a fault, possibly due to a blockage in the probe and tubing assembly or fluid link tubing. For functionality the sample tubing needs to be pushed firmly into the clamp during installation and requires re-calibration after a probe and tubing change. It is suggested that the calibration is executed in a consistent lab environment, for example, the lighting levels should match as those used during general instrument running.

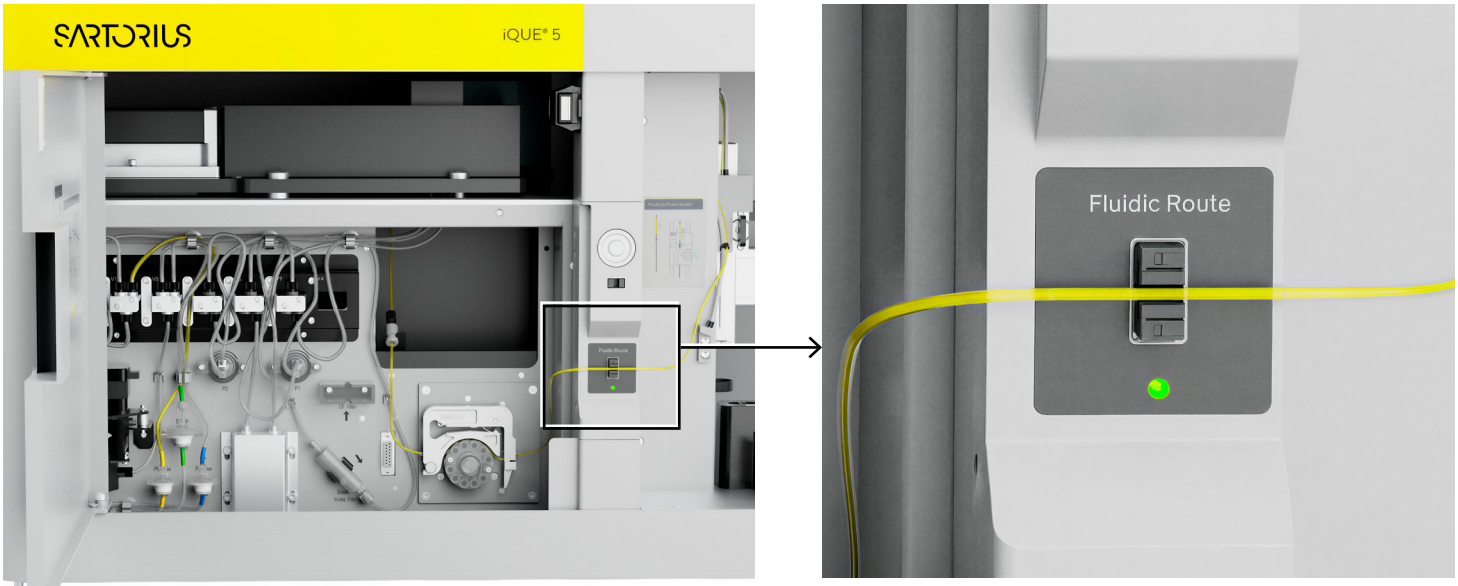


Figure 2: Position of the Bubble Sensor Indicator

iQue® 5 Clog Detection Feature

Within the iQue Forecyt® Software users can choose to run protocols with or without enabling the clog detection feature on the iQue® 5 instrument. This can be set up as part of the plate acquisition controls and utilizes features of the bubble frequency and timing as an indicator for disrupted flow (Figure 3). This disruption is measured as a combination of changes to the frequency of the bubbles passing the sensor, fragmented bubbles within the fluid phase or deviations from the number of bubbles expected.

The sensitivity of detection can be set at varying levels depending on user requirements or the samples being tested. A guide to the sensitivity is shown in Table 1, with the default sensitivity meaning a clog is flagged when <75% bubble integrity is detected, or when 5-7 wells are missing.

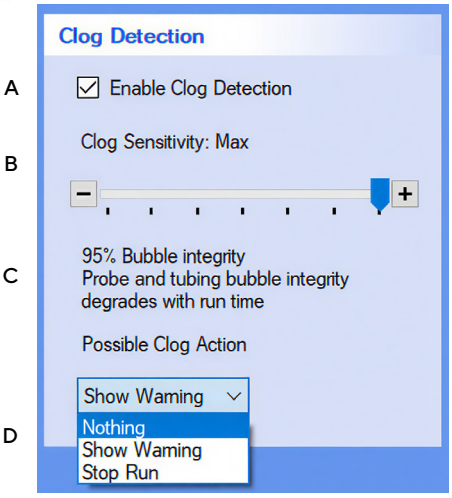


Figure 3: Enable Clog Detection Within Plate Acquisition Protocol.
A – turn clog detection on/off, B – Sensitivity slider, C- confidence level indicator, D – Action if clog detected

Sensitivity	Description	Bubble Integrity *or*	Missed Wells
Max	Any interruption, serious clog auto stop run	<95%	1-2
Very High	Any clog	<90%	2-3
High	Light clog	<85%	3-5
Average (default)	Clog	<75%	5-7
Low	Serious clog needed	<65%	7-9
Very Low	Egregious clog needed	<50%	9-11
None	No clog detected	–	–

Table 1: Detection Sensitivity Guidelines

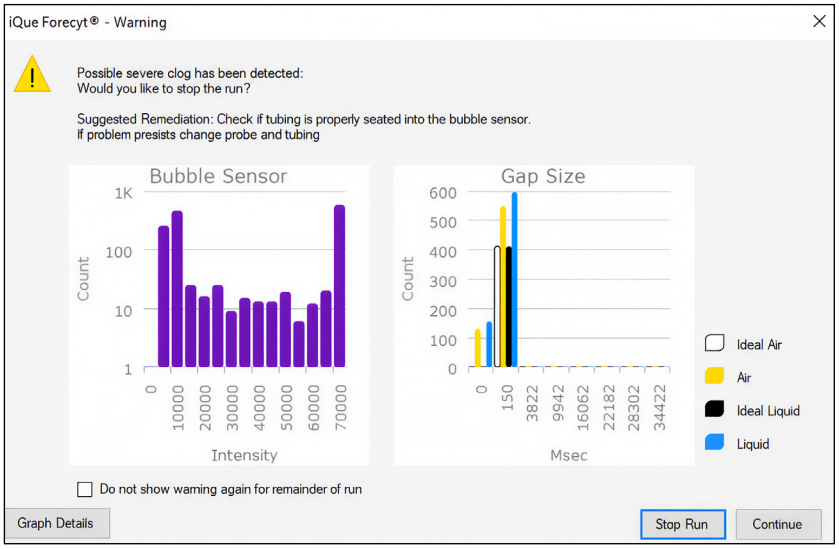
When a clog is detected, the user can set an action from the drop-down list:

- **Nothing** – ignore any detection and continue sample acquisition
- **Show Warning** - display a warning on the screen with associated details of what has been detected (see details below) but do not stop the run.
- **Stop Run** - automatically stop acquisition.

When a warning is triggered, the instrument will display it on the screen. The run will only stop if "Stop Run" is selected, otherwise sample collection will continue. Along with the warning, a set of graphs will be displayed.

- The warning (Figure 4A) provides information linked to the detected issue and offers options to "Stop Run" or "Continue". Data from the plate run is displayed as two graphs for bubble sensor and gap size. The count indicates the number of wells in the plate counted. If early in the plate run, this number will be low and more sensitive to data fluctuations. By clicking the button "Graph Details", it is possible to see examples of good and bad runs to compare datasets.
- The left-hand graph (Good) in Figure 4B demonstrates how bubble sensor peaks should ideally show a split between low and high values. If disrupted or "shredded" bubbles or unexpected bubble sizes are detected there will be no clear split in populations (right-hand graph in Figure 4B, 'Bad').
- Figure 4C shows examples of good and bad graphs describing the gap sizes for both the air gap and sample slugs. It is the difference between the ideal gap data and the measured gap data that is important. The sample gap size will be dependent on the sip time as highlighted in the two sets of graphs (Figure 4C).

A



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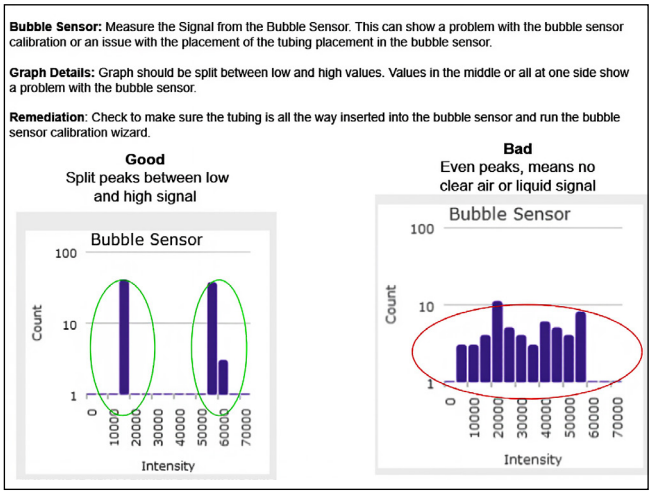

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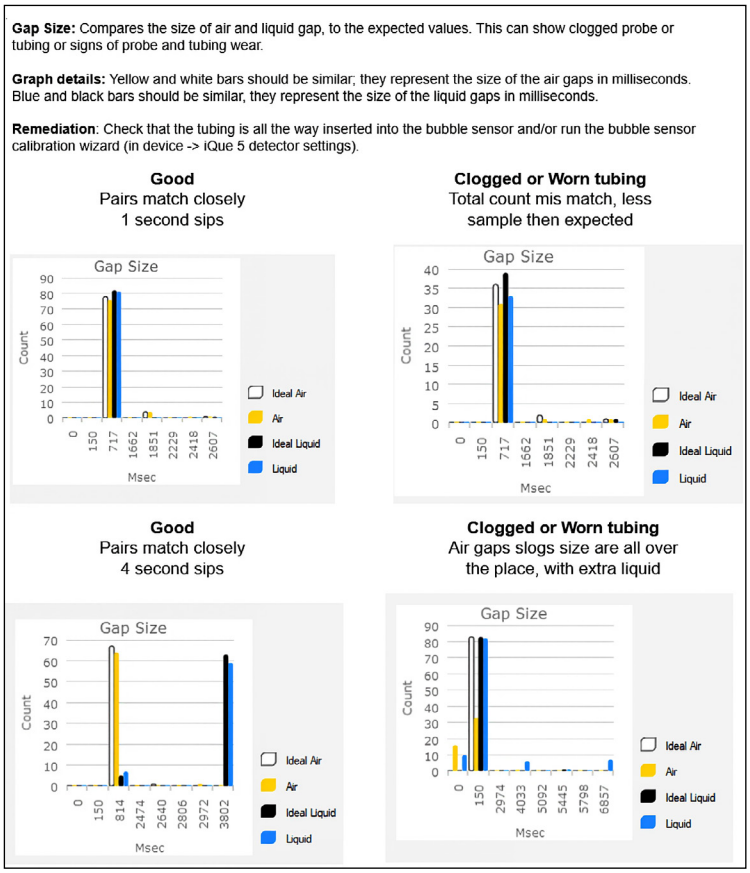

Figure 4: Information Display When Potential Clog Warning Is Detected.
(A) Graphical display of detected issue on current plate showing bubble sensor and air gap details. (B) and (C) examples of "good" and "bad" data for both bubble sensor and gap size.

Other Features

Under the Device tab there are a couple of additional tools related to the clog detection and management feature (Figure 5).

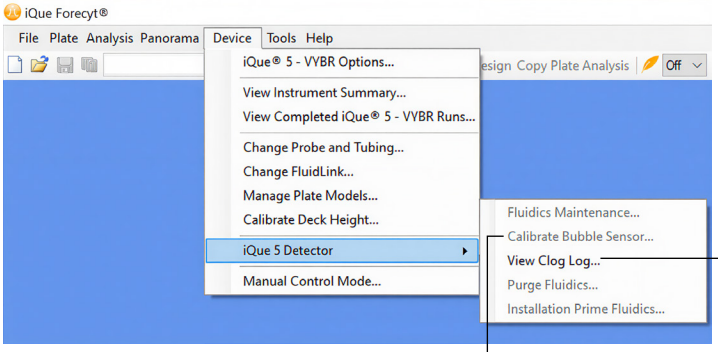

Figure 5: Other Clog Detector Features

Calibrate Bubble Sensor

It is recommended to run this calibration after a probe and tubing change and if there are excessive clog warnings but no visible issue with the bubble in the tubing. This is an easy-to-follow wizard.

View Clog Log

After each completed plate, Forecyt will store all details associated with the plate (Table 2). The data collected summarizes information from the run settings, any warnings detected and details on the warning trigger. In Table 2, the first plate, Example A shows "No Clog", but the second plate, Example B was flagged for "Air Gap Count Mismatch". The data supporting this can be seen in bubble counter column where an extra 81 bubbles were detected (highlighted). This plate also suffered from "Shredded Bubbles", but the data capture was completed with no loss in wells. More drastic effects on air gap quality will result in reduced sampling and poorer well identification. Information from the log is stored on the device and can be exported as a CSV file if required for further processing.

Type	Plate: Example A Medium (Default)	Plate: Example B Medium (Default)
Clog Details	Threshold: Medium (Default) No Clog	Threshold: Medium (Default) Warning Detected at 514.9s
Date	13/08/2025 10:46:06	13/08/2025 13:51:51
Bubble Intensity	Bubble intensity distribution score: 100 Bubble sensor histogram: 2 bins have at least 5% of total count. Low range bins: 1, High range bins: 1	Bubble intensity distribution score: 100 Bubble sensor histogram: 2 bins have at least 5% of total count. Low range bins: 1, High range bins: 1
Bubble Counter	Air Gap Counts = 100% Air Bubbles = 0 Measured/expected 432/432	Air Gap Counts = 118.66% Extra Gaps Air Bubbles = +81 measured/expected 515/434
Bubble Air and Liquid Time Comparer	Air Gap Length Quality = 93.75% More Liquid Air Diff = -32.251s 407.408s/439.659s Liquid Diff = +28.272s 575.86s/547.588s Total Diff = -3.979s 983.268s/987.247s	Air Gap Length Quality = 97.25% More Liquid Air Diff = -14.817s 423.429s/438.246s Liquid Diff = +11.665s 560.833s/549.168s Total Diff = -3.152s 984.262s/987.414s
Concordance Analyzer	Gap Size Histogram Score: 99.94 Liquid score 100.0 Air score: 99.9	Gap Size Histogram Score: 94.38 Liquid score 91.4 Air score: 97.3
Shredded Bubble Counter	Shredded Bubbles = 0 Shredded Bubbles Diff Measured/expected = 0: 0/0	Shredded Bubbles = 40 Shredded Bubbles Diff measured/expected = +40: 40/0
Tags	None	Airgap Count Mismatch

Table 2: Example of "Clog Log" Details

Case Study Data — iQue®5 HTS Platform

To test the detection and effect on well identification, a 384 well plate was set up containing unhealthy Jurkat cells to represent a sticky cell sample. The plate was tested under various settings to look at the effect in the "Clog Log". The "Clog Log" data is shown in Table 3 for three plates:

- **Plate A** — Collected with no inter-well shaking or washes on a high sensitivity setting. Several warnings were detected linked to air gap quality (number, shredded bubbles, highlighted in Table 3). Looking at the well identification time histogram, the software has detected 384 wells, but the quality of the sample slugs and air gaps is poor and is decreasing in quality as the plate proceeds. There are still events being collected so this is not a clog, but the quality of data collection is reduced (Figure 6A).
- **Plate B** — Collected directly after plate A but with the introduction of shaking and washing steps after every 24 wells (at the end of each row). This was also collected on a high sensitivity setting. With the changes to the protocol, the warnings were not seen and well ID was improved, reflective of the even sample and air gap quality (Figure 6B).
- **Plate C** — Collected after several other plates with no inter-plate washes. This resulted in multiple warnings and a partial clog. The major warnings on this plate were both extensive effects on air gap count (50%) and air gap quality (9% highlighted in Table 3). The well identification time histogram (Figure 6C) shows a decrease in the event counts across the plate.

Type	Plate A Jurkat High	Plate B Jurkat High with wash/shake	Plate C Jurkat Very High NO wash
Clog Details	Threshold: High Warning	Threshold: High No Clog	Threshold: Very High Warning
Bubble Intensity	Bubble intensity distribution score: 50 Bubble sensor histogram: 3 bins have at least 5% of total count. Low range bins: 0, High range bins: 3	Bubble intensity distribution score: 100 Bubble sensor histogram: 2 bins have at least 5% of total count. Low range bins: 1, High range bins: 1	Bubble intensity distribution score: 100 Bubble sensor histogram: 2 bins have at least 5% of total count. Low range bins: 1, High range bins: 1
Bubble Counter	Air Gap Counts = 182.45% Extra Gaps Air Bubbles = +357: Measured/expected 790/433	Air Gap Counts = 99.64% Air Bubbles = -2: measured/expected 553/555	Air Gap Counts = 50.45% Missing Gaps Air Bubbles = -219: Measured/expected 223/442
Bubble Air and Liquid Time Comparer	Air Gap Length Quality = 96.49%	Air Gap Length Quality = 91.13%	Air Gap Length Quality = 9.37%
Concordance Analyzer	Gap Size Histogram Score: 73.53 Liquid score 70.8, Air score: 76.3	Gap Size Histogram Score: 99.05 Liquid score 98.9, Air score: 99.2	Gap Size Histogram Score: 51.38 Liquid score 40.7, Air score: 62.0
Shredded Bubble Counter	Shredded Bubbles = 140 Shredded Bubbles Diff: Measured/expected = +140: 140/0	Shredded Bubbles = 4 Shredded Bubbles Diff: Measured/expected = +4: 4/0	Shredded Bubbles = 24 Shredded Bubbles Diff: Measured/expected = +24: 24/0
Tags	FlatlineAir, MildShredding, SevereShredding, BubSenDistrib, BubSenLows, DiscordantAir, DiscordantLiquid, AirgapCountMismatch	None	MildShredding, SevereShredding, DiscordantAir, DiscordantLiquid, AirgapCountMismatch

Table 3: Clog Log Information for 3 Test Plates

Plate A

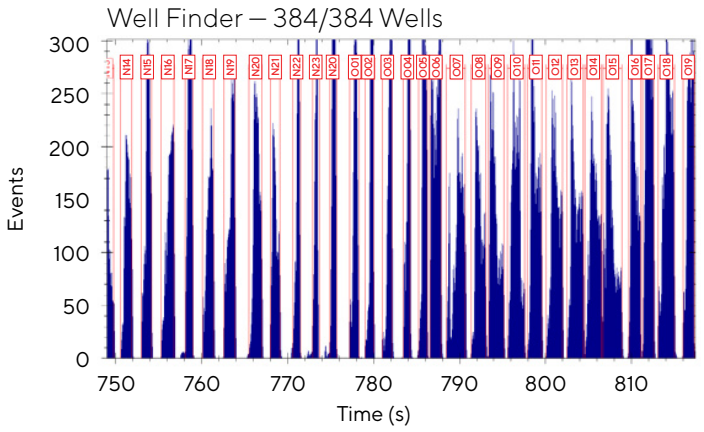

Plate B

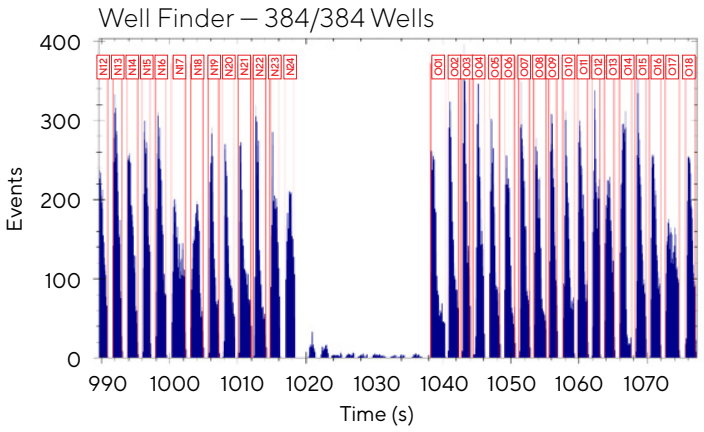

Plate C

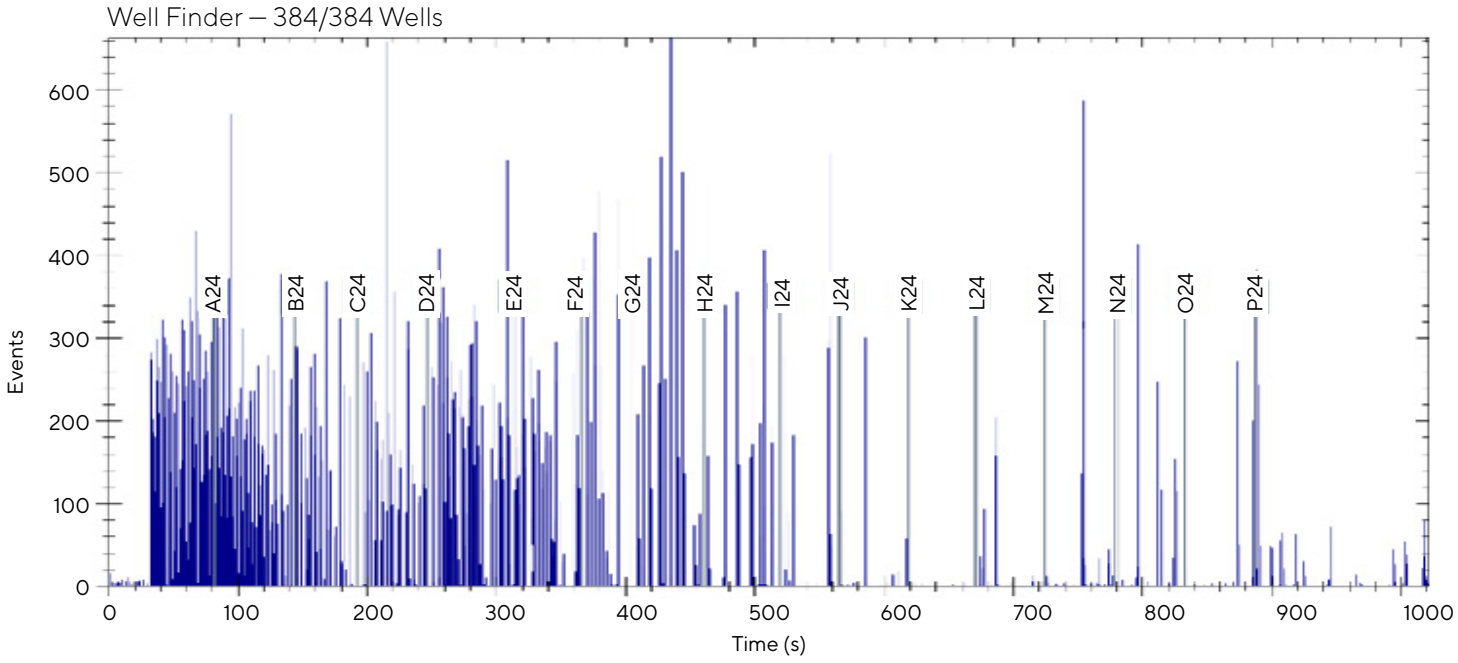

Figure 6: Well identification time histograms for plate A, B and C.

Instrumentation Practices

Improved clog detection is one of multiple tools available to aid users in reducing the frequency of clogging in cytometry. Check out a previous tech note in this area for other suggestions to help **prevent clogs**. In addition to clog detection, other best practices to keep your instrument in good working condition include:

- Correct start-up and shut-down before and after use.
- Following daily, weekly, and monthly schedules for cleaning and maintenance.
- Running daily QC to monitor instrument performance.
- Adhering to recommended annual preventative maintenance visits.

There are various cleaning protocols within the iQue Forecyt® Software which are listed in Table 4. The software will automatically alert the user when these are due and there are easy to follow wizards to guide you through the process. These procedures can be performed more frequently if needed based on usage and sample types. All the standard cleaning procedures are accessed in the controller console window through the Clean Wizard. This is supported by a cleaning log which provides a list of the procedures that have been performed, showing the date, time, and user who performed the cleaning. Working with samples that are prone to aggregation may require more frequent cleaning and maintenance.

Frequency	Procedure	Comments
Daily	Probe and Tubing Clean	Performed as part of both instrument startup and shutdown. This runs a sequence of Flush, Clean and Rinse using the solutions on the sampling deck.
	Daily QC	Performed after startup and before running samples. Ensures the system performance is within specification and is also a useful diagnostic tool if issues arise.
Weekly	Change Probe and Tubing	Users will be automatically notified when the recommended interval for change occurs. Automatic warning will display when due.
	Detector Long Clean	Perform as part of end-of-week shutdown procedure.
Monthly	Fluidics Maintenance	Automatic notification of maintenance will indicate when and how to perform fluidics maintenance. Automatic warning will display when due. There are several parts that are designed to be regularly replaced including various filters and the FluidLink.
	Detector Unclog	Performed as a preventative clean.
On Demand	Detector Quick Clean	Performed as needed to clean the instrument. Suggested to be used if the instrument has been idle for a short time.
	Detector Long Clean	Performed as needed to clean the instrument. Use after heavy usage or a long period of inactivity.
	Detector Unclog	Performed as needed to address a clog.
	Detector Debubble	Performed as needed to clear bubbles. Use if Daily QC shows irregular scatter, after fluidics maintenance, and when fluids are added to the bottles.

Table 4: Overview of Recommended Maintenance

Probe and Tubing

Changing the probe and tubing is a quick and easy way to remove a stubborn clog. As the iQue® Platform runs, the main sample tubing can be worn by the rollers of the peristaltic pump. To ensure proper sample collection, it is recommended to change the assembly when prompted by iQue Forecyt® but, if issues arise, the assembly should be changed as needed. A dedicated wizard is available to guide you through this procedure, located on the device tab.. A new feature on the iQue® 5 Platform: the pump clamp will automatically open when the cytometer is powered off and close when the instrument is started up.

Summary

This technical note provides an overview of the new clog detection and management features of the iQue® 5 HTS Platform and iQue Forecyt® Software suite. powered off and close when the instrument is started up.

Glossary of Warning Terms

- **AirgapCountMismatch** —Mismatch in the number of measured and expected air gaps.
- **BubSenDistrib | BubSenLows** — Generated from the bubble sensor histogram and indicates an uneven spread of high and low peaks on the histogram, suggestive of uneven air gaps.
- **FlatlineAir | FlatlineLiquid** — Bubble Count favors either air or liquid, suggestive of a clog or worn probe and tubing.
- **DiscordantAir | DiscordantLiquid** — Total time favors either air or liquid, suggestive of a clog or worn probe and tubing.
- **MildShredding | SevereShredding** — Linked to number of shredded bubbles detected.

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