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Performance Evaluation of Flownamics Sampling Devices for the Agilent Online LC

Combination of the Agilent 1260 Infinity II Online LC with Flownamics SegFlow S3 and SampleMod

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Abstract

This technical overview demonstrates how an Agilent 1260 Infinity II Online LC can be used in combination with the Flownamics SegFlow S3 and SampleMod modules for sampling from a bioreactor. The plumbing, electronics, and software setup are described, and some critical performance data are measured and discussed. This combination enables automated sampling and dilution from a bioreactor in an unattended manner for continuous monitoring of biologic reactions.

Introduction

Today, modern biopharmaceuticals, such as monoclonal antibodies, are typically produced by living cells in bioreactors. To optimize the production and the quality of growth media, it is increasingly advantageous to draw samples from the bioreactor under aseptic conditions using specialized sampling devices. For the measurement of the expected product, the sample can be diluted and transferred to a 1260 Infinity II Online LC.

This technical overview describes the combination of the Agilent 1260 Infinity II Online LC with the Flownamics SegFlow S3 and SampleMod modules for automated bioreactor sampling. A description of the plumbing, electronics, and software setup is given. Some critical performance data, such as dilution precision, linearity, and carryover between reactors are measured and discussed.

Experimental

The instrumentation used in this study is detailed in Table 1, and the method parameters are outlined in Tables 2 and 3.

Table 1. Instrumentation.

Product Type	Agilent Product Description
Instrument	− Agilent 1290 Infinity II High-Speed Pump (G7120A) − Agilent 1260 Infinity II Online Sample Manager Set (G3167AA): Agilent 1260 Infinity II Online Sample Manager (G3167A) clustered with external valve (p/n 5067-6680) located at the Agilent 1290 Infinity Valve Drive (G1170A), and Agilent Online LC Monitoring Software − Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) − Agilent 1290 Infinity II Diode Array Detector (G7117B) with Agilent InfinityLab Max-Light Cartridge Cell (10 mm, p/n G4212-60008)
Additional Hardware	− Flownamics SegFlowS3: Automated sampler for integration to process analytic devices* − Flownamics Sample-Mod S3: For sample pre-dilution and distribution to the analyzers*
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 3.0 × 50 mm, 1.8 µm (p/n 959757-302)
Software	− Agilent OpenLab CDS, version 2.6 or later − Agilent Online LC Monitoring Software, version 1.2 − FlowWeb (control software, Flownamics)

* Refer to Flownamics for part numbers

Table 2. Method parameters.

Parameter	Value
Analytical Method Conditions	
Solvents	A) Water + 0.1% formic acid (FA) B) Acetonitrile (ACN) + 0.1% FA
Analytical Flow Rate	1.0 mL/min
Solvent Composition	Isocratic 15% B
Column Temperature	45 °C
Feed Speed	80% of analytical flow rate
Flush-Out Solvent	Water:ACN 9:1 + 0.1% FA (S2)
Flush-Out Volume	Automatic
Injection Volume	1 µL
Needle Wash	3 s, water:ACN 1:1 + 0.1% FA (S1)
Diode Array Detector	273 ± 4 nm, reference: off, 20 Hz data rate
Online LC Monitoring Software	
Sampling Modes	Direct injection Dilute to vial Pure to vial
Draw Speed	Setting 2 − Draw speed: 100 µL/min − Wait time: 3.6 s
Schedule	Not necessary, controlled by the Flownamics software

Table 3. Flownamics software and hardware settings.

Flownamics Software Settings for SegFlow S3	
Dilution	1:1, 1:2, 1:5, 1:10, 1:20
Purge and Sample Volume	0.5 and 4.0 mL
Frequency	Every 30 min
Repeats	Typically 10
Tubing Length to ASR	10 ft (3 m)
Flownamics Software Settings for SampleMod	
Presample Volume	0.5 mL
Sample Volume	3 mL
Flownamics Hardware	
Solvents at SegFlowS3 and SampleMod	SAN (sanitizer, iPrOH 20%) DET (detergent, water) DI (de-ionized water) DIL (diluent, water)

Instrument setup

The electronic and flow path connection setup from a bioreactor to the Agilent 1260 Infinity II Online LC through the Flownamics sampling device is shown in Figure 1. The Online LC and the Flownamics SegFlow S3 are connected to a PC by LAN and a hub. The Flownamics SegFlow S3, SampleMod, and ASR (Aseptic Sample Regulator) are connected as a system to each other. The flow path leads from the ASR to the SegFlow S3, then to the SampleMod, and finally to the reactor interface valve of the Agilent 1260 Infinity II Online LC. Solvents for cleaning are connected to the SegFlow S3 and the SampleMod. Ambient air can be drawn at the ASR and the SegFlow S3.

Sampling process

The sampling from a bioreactor is done by the Flownamics In-situ Sampling Probe (FISP), which allows for cell-free and cell-containing samples as needed. The probe is directly connected to the ASR, which is located at the bioreactor. The ASR is connected to the SegFlow S3 by a specially configured sterile tubing set. The ASR comprises several valves and flow sensors to regulate the sample flow from the reactor to the SegFlow S3. It also regulates the cleaning fluid flow from the SegFlow S3 to the ASR.

The flow at the SegFlow S3 is controlled by an inherent peristaltic pump. Up to eight different reactors can be connected (one ASR and one FISP each) with a distance of 3 or 7 m (according to the preconfigured tubing and settings in the software). The sample is positioned by air gaps controlled by fluid/air sensors at the ASR, the SegFlow S3, and the SampleMod. The air gaps are set at the ASR and allow flexible distances between SegFlow S3 and the SampleMod, as well as the SampleMod and the 1260 Infinity II Online LC. Under this control, the sample plug is handed over to the Flownamics SampleMod for dilution.

After sample collection in a sample cup, the appropriate aliquots are mixed in a sample dilution cup using a syringe. This syringe is also used to send the sample plug to the reactor interface valve on the 1260 Infinity II Online LC. The syringe allows pressure resistance on the way to the Online LC interface to be overcome. The arrival of the sample plug at the Online LC interface is also controlled by a liquid/air sensor, located between the Online LC interface and the waste bin.
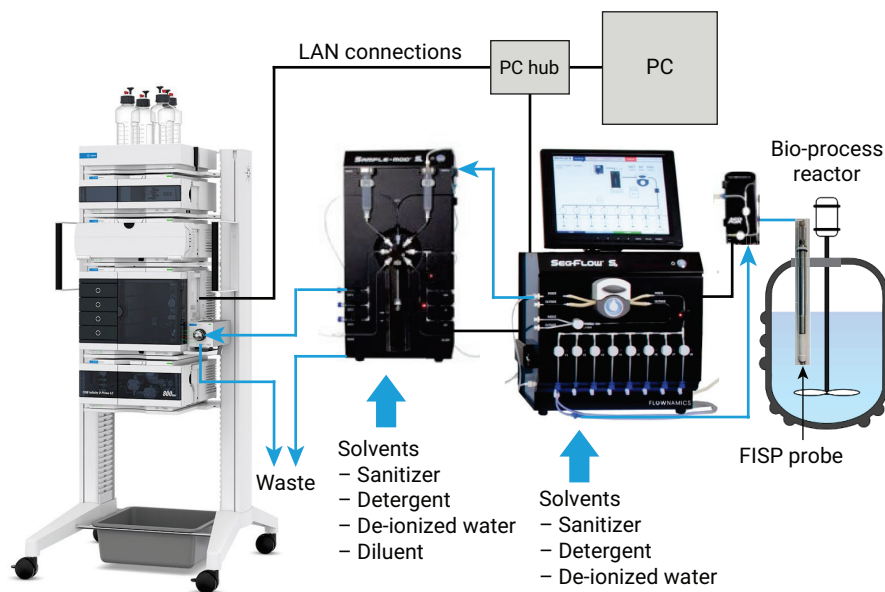

Figure 1. Setup of the Agilent 1260 Infinity II Online LC, together with the Flownamics S3 and SampleMod (black: electrical connections, blue: flow connections).

Software setup

– **Agilent OpenLab CDS:** The acquisition and data analysis methods are run by OpenLab CDS. The CDS must be released for external control before the experiment starts.

– **Agilent Online LC Monitoring Software:** An experiment method is set up by choosing the acquisition method and the data analysis method created previously in OpenLab CDS. For sampling, **Direct Injection**, **Dilution to Vial**, or **Pure to Vial** can be selected. The experiment setup must not contain more than one sampling setting, but can also contain blanks and quality control samples. The schedule must not contain any time-based entries.

– **Flownamics control software:** To enable communication between the Flownamics control software and the Agilent Online LC Monitoring Software, the Web API (application programming interface) must be started. This can be done using Microsoft Windows services. The main screen of the Flownamics control software, which is accessed after user login, is shown in Figure 2. From this screen, the selection of the desired bioreactor vessel leads to the standard controls. These will start or stop the sampling and enable the general values for the experiment to be entered (Figure 3A). More details can be entered in the Settings tab (Figure 3B).
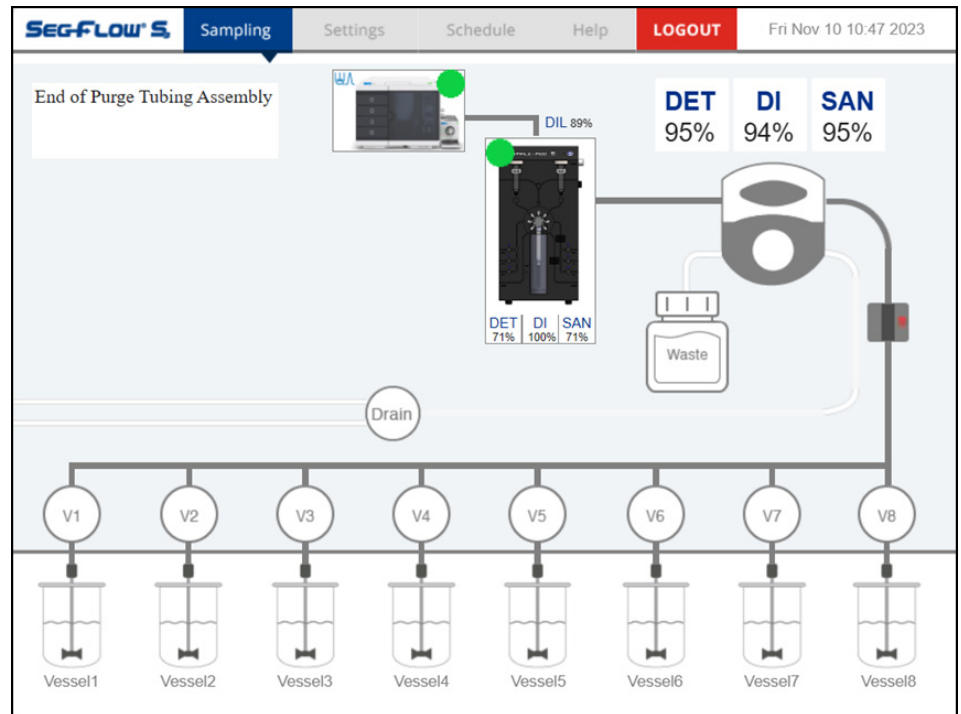

Figure 2. The main screen of the Flownamics control software, FlowWeb, with control access to eight individual bioreactors. The SegFlow S3 is symbolized by the peristaltic pump, the SampleMod is shown by an instrument photo, and the Agilent 1260 Infinity II Online LC is symbolically shown as the Online Sample Manager with a sampling interface valve. The individual filling of solvent bottles is expressed as percent value.
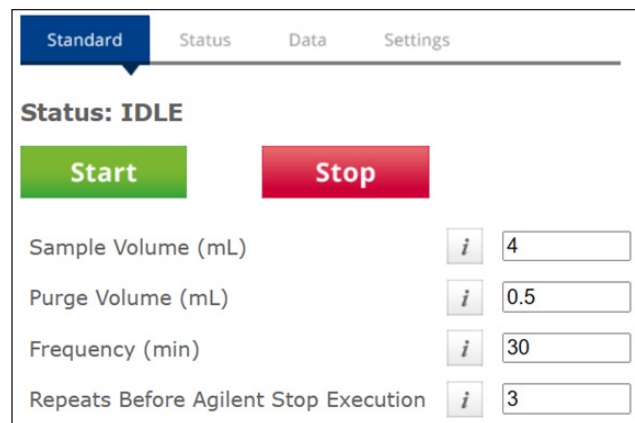

Figure 3A. Standard reactor control settings, accessible for each connected bioreactor vessel.

Figure 3B. Detailed settings for each employed bioreactor, specifying the conditions for the connected Agilent Online Monitoring Software and Agilent Online LC.

The Sample Setting, which is set up in the Online Monitoring Software, can be selected as **Agilent InfinityLab Experiment**. If a **Direct Inject** setting is selected, no information is needed about vial positions in Agilent Vial Settings. If a **Dilute to Vial** or a **Pure to Vial** setting is chosen, the vial positions for sampling must be entered here in JavaScript Object Notation (JSON) format (see Appendix). For an additional blank injection from a vial or QC sample, the JSON script can be modified (see Appendix). To include the Agilent Online LC, the appropriate Agilent InfinityLab settings must be selected.

In the InfinityLab settings, the dilution rate can be set (a dilution rate of 1:1 means that undiluted sample will be sent to the Agilent Online LC). In Figure 3B, the tubing length between the ASR and SegFlow S3 is set to 10 ft (3 m).

Selecting the **Sample Mod** enables settings for Agilent InfinityLab Presample Volume and Agilent InfinityLab Sample Volume, which define the volumes finally sent to the Agilent Online LC interface valve.

Sample
Caffeine: 1 and 15 g/L in water

Solvents and chemicals
- All solvents were purchased from Merck, Germany.
- Chemicals were purchased from VWR, Germany.
- Fresh, ultrapure water was obtained from a Milli-Q integral system, equipped with an LC-Pak polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

Results and discussion

When evaluating the performance of the Flownamics SegFlow S3 and the Flownamics SampleMod, in combination with the Agilent Infinity II Online LC, three performance parameters were reviewed. The peak area precision was determined for different dilution and sampling modes available in the Online Monitoring Software. For dilution using the Flownamics SampleMod, the linearity was determined. Finally, carryover from the sampling in one reactor to another was determined.

To establish the peak area precision of dilution using the SampleMod, followed by a direct injection from the Online LC, the dilution factors of 1 (undiluted), 2, 5, 10, and 20 were applied. The reactor sample (1 g/L caffeine solution) was transferred to the sampling cup at the SampleMod and then mixed with the respective volume of diluent solution. The obtained diluted reactor solution was transferred to the Online LC interface valve and injected directly. The peak area precision, expressed as relative standard deviation (RSD), obtained for 10 replicates each, was typically between 0.4 and 0.8% (Figure 4, blue bars).

Dilutions with dilution factors higher than 20 are not possible using the SampleMod, but can be done directly through the Online LC. For this application, the undiluted reactor sample can be directly handed over to the Online LC interfacing valve by the SampleMod.

The dilution was done completely within the Online LC Sample Manager, followed by injection. Typical peak area precision was between 0.4 and 0.7% RSD (Figure 4, orange bars). Dilutions below a factor of 10 should be done by the SampleMod, as it can achieve better precision values for lower dilution factors. To retain samples of the lower dilution factors, the

"Pure to Vial" sampling function on the Online LC was tested, too. In this case, the sample diluted by the SampleMod was collected in a vial in the Online Sample Manager, followed by injection. The precision was typically between 0.4 and 1.0% RSD (Figure 4, gray bars).

For the determination of dilution linearity using the SampleMod, the peak areas obtained for dilution factors of 1, 2, 5, 10, and 20 were plotted over the calculated concentration of each dilution (Figure 5). With an R^2 of 0.9992, the obtained linearity was excellent for the examined SampleMod dilution range.

The obtained area precision and linearity values were in accordance with values determined earlier for the Online LC Sample Manager.[1]

The discussed area precision (Figure 4) describes the performance of repeatability, but does not describe the accuracy. The accuracy describes how close a measured value is compared to an expected value. For the determination of accuracy, a caffeine solution (1.0 g/L in water) was used. The expected values were determined from a series of handmade dilutions (undiluted, 1:2, 1:5, 1:10, and 1:20) and injected 10 times.
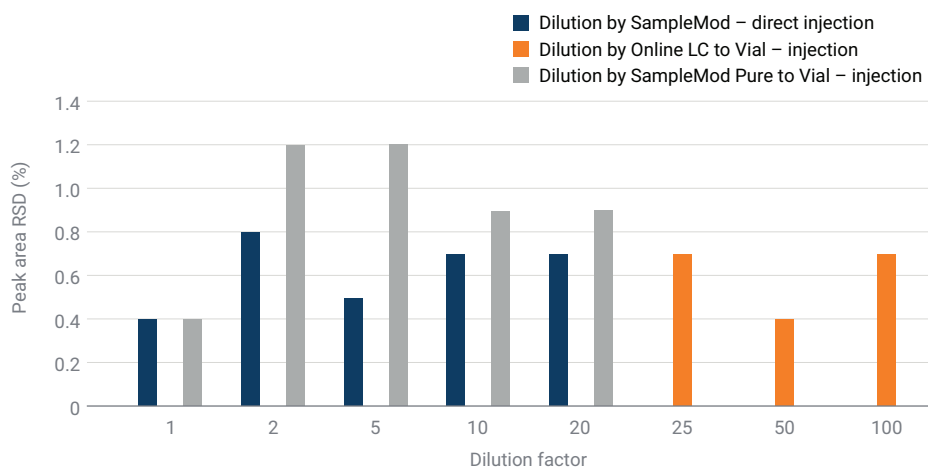

Figure 4. Peak area RSD values (n = 10) obtained from dilution by SampleMod, followed by direct injection (blue), dilution to a vial by Online LC then injection (orange), and dilution by SampleMod with Online LC Pure to Vial sampling then injection (gray).
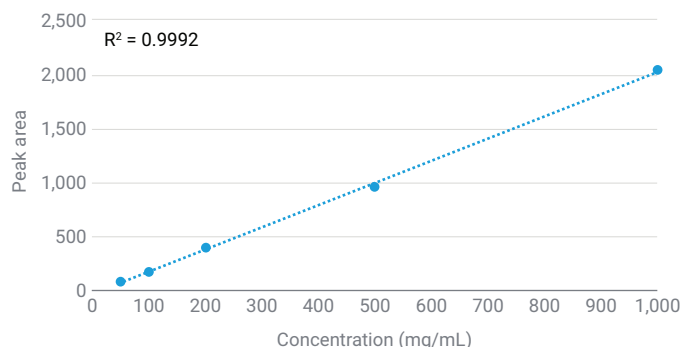

Figure 5. Linearity of dilutions, obtained by mixing using the SampleMod with dilution factors of 1, 2, 5, 10, and 20.

An identical dilution series was measured using the Flownamics S3 with connected SampleMod. For the preparation of the dilution series, the SampleMod uses a step motor-driven syringe, which was operated using the default values. When the undiluted caffeine solution was sent to the sampling interface valve of the Online LC, an accuracy reading of 91% was measured, in comparison to the peak areas directly injected from a sample vial in the LC. The other dilutions showed an accuracy of 81 to 88% (Figure 6). These values could be optimized by changing the values of the syringe motor steps, and providing more or less diluent.

The Flownamics S3 module can connect and handle up to eight reactors. As these reactors use the same flow path through the S3 module to the SampleMod and then to the Online LC interface valve, carryover from one reactor content analysis to the next is possible. To test the Flownamics sampling device for carryover, a second ASR was connected to the S3 module. The first reactor vessel was loaded with a highly concentrated caffeine solution (15 g/L) and the second with pure de-ionized water. Three sampling cycles were done from the vessel containing the highly concentrated caffeine solution. These samples were diluted 1:20 at the SampleMod and sent to the Online LC interface valve. The dilution needed to be in the linear range of the applied diode array detector for peak area detection to calculate the carryover. The following sample cycle was done from the second vessel, containing pure water. In this instance, a carryover of 0.44% was measured (Figure 7). In contrast, the typical carryover of the Online LC Sample Manger for injections between different vials is below 30 ppm.[2]
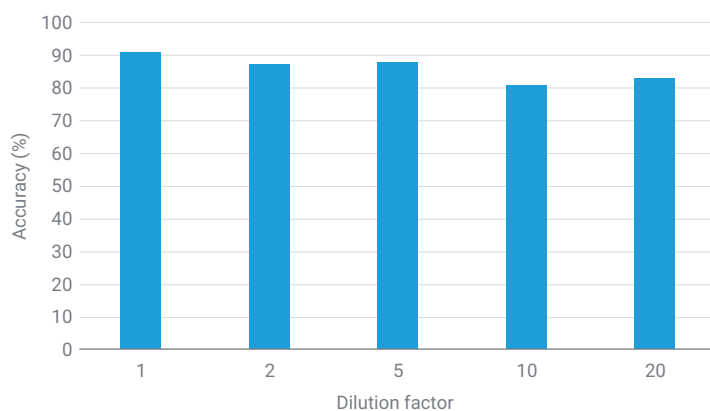

Figure 6. Accuracy of dilutions made using the Flownamics SampleMod, applying default conditions for the syringe step motor.
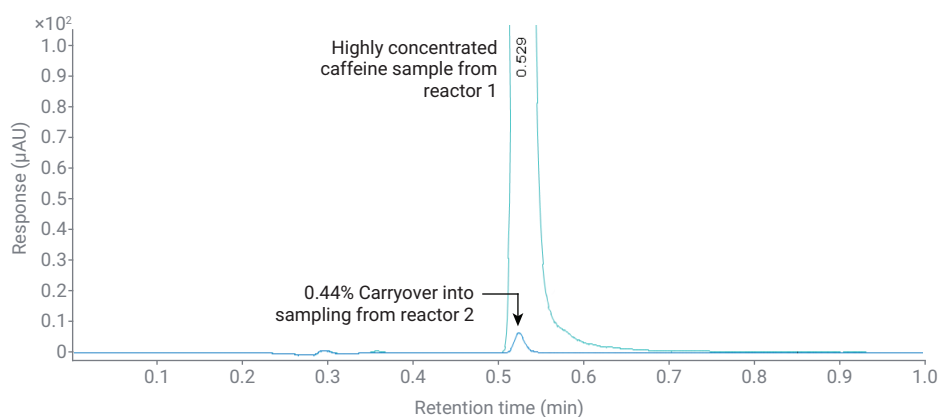

Figure 7. Determination of carryover from the content of reactor 1 into the analysis of the reactor 2 content.

Conclusion

This technical overview describes the setup of an Agilent 1260 Infinity II Online LC with Flownamics S3 and SampleMod for sampling from a bioreactor. For a complete setup, the most important performance parameters, such as peak area precision with and without dilution (in combination with different sampling modes), accuracy, dilution linearity, and carryover were measured. The obtained values were all in a good range, as expected for the applied setup.

References

1. Herschbach, H.; Kutscher, D.; Naegle, E.; Ortmann, T.; Wouters, S. Reactor Sample Dilution and Mixing Performance of the Agilent 1260 Infinity II Online Sample Manager. *Agilent Technologies technical overview*, publication number 5994-3679EN, **2021**.

2. Herschbach, H.; Kutscher, D.; Naegle, E.; Ortmann, T.; Spuling, I.; Wouters, S. Performance Characteristics of the Agilent 1260 Infinity II Online Sample Manager. *Agilent Technologies technical overview*, publication number 5994-3529EN, **2021**.

Appendix

Examples of the JSON format, which is a standard text-based format representing structured data, based on the JavaScript object syntax for Agilent Vial Settings. These text files can be generated by exporting the selected vial positions from the Online Monitoring Software to a .txt file.

Example 1

Format for a Diluted to Vial method. Locations are in drawer 1, back, wells B1 to B10.

```
{
  "Locations": [
    {
      "Name": "DilutedToVialSetting 01",
      "SourceLocation": "",
      "TargetLocations": [
        "D1B-B1",
        "D1B-B2",
        "D1B-B3",
        "D1B-B4",
        "D1B-B5",
        "D1B-B6",
        "D1B-B7",
        "D1B-B8",
        "D1B-B9",
        "D1B-B10"
      ]
    }
  ]
}
```

Example 2

Format for a Diluted to Vial method. Locations are in drawer 1, back, wells B1 to B10, and a vial for a blank injection is in position 1.

```
{
  "Locations": [
    {
      "Name": "BlankSampleSetting 01",
      "SourceLocation": "1"
    },
    {
      "Name": "DilutedToVialSetting 01",
      "SourceLocation": "",
      "TargetLocations": [
        "D1B-B1",
        "D1B-B2",
        "D1B-B3",
        "D1B-B4",
        "D1B-B5",
        "D1B-B6",
        "D1B-B7",
        "D1B-B8",
        "D1B-B9",
        "D1B-B10"
      ]
    }
  ]
}
```

Example 3

Format for a Diluted to Vial method. Locations are in drawer 1, front, wells B1 to B10. There is also a vial for a blank injection in position 1, and a vial for a QC in position 2.

```
 "Locations": [
   {
     "Name": "BlankSampleSetting 01",
     "SourceLocation": "1"
   },
   {
     "Name": "PureToVialSetting 01",
     "TargetLocations": [
       "D1F-B1",
       "D1F-B2",
       "D1F-B3",
       "D1F-B4",
       "D1F-B5",
       "D1F-B6",
       "D1F-B7",
       "D1F-B8",
       "D1F-B9",
       "D1F-B10"
     ]
   },
   {
     "Name": "QCSampleSetting 01",
     "SourceLocation": "2"
   }
 ]
}
```

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