Cell Culture Monitoring with the Vi-CELL MetaFLEX

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INTRODUCTION

A common goal in the Biologics industry is to produce the best quality product at the highest yield. To achieve this, it is necessary to control and monitor the environment in bioreactors. The Vi-CELL MetaFLEX can be applied to achieve these goals. The Vi-CELL MetaFLEX is an automated bioanalyte analyzer used for analyzing mammalian and insect cell culture media. It measures the parameters most important in cell culture: pH, Glucose, Lactate, pCO_2, pO_2, and electrolytes. Based on Radiometer[1]'s leading point-of-care blood gas analyzer technology, the MetaFLEX is a powerful technology for the measurement of take action parameters that impact cell viability.

The MetaFLEX analyzer requires only a 65µL sample and provides results in 35 seconds. Samples can be introduced to the system via syringe, capillary tube, test tube, sample cup, and similar containers. The sample is aspirated from the sample container by the analyzer's inlet probe, and testing begins immediately. The sample is passed through a replaceable sensor cassette where miniaturized sensors take measurements. All of the measurements performed by the MetaFLEX are conducted within the sensor cassette, and all necessary fluids are contained within a replaceable solution pack. The solution pack also contains the solutions required to perform internal quality control and calibrations, as well as a container for waste. The user will be notified by the analyzer when these consumables need to be replaced due to reaching their maximum number of tests and/or their on-board expiration date.

METHODS OF DETECTION

The sensor cassette used in the MetaFLEX contains sensors/electrodes for each of the parameters measured, including a reference electrode (see Figure 1).

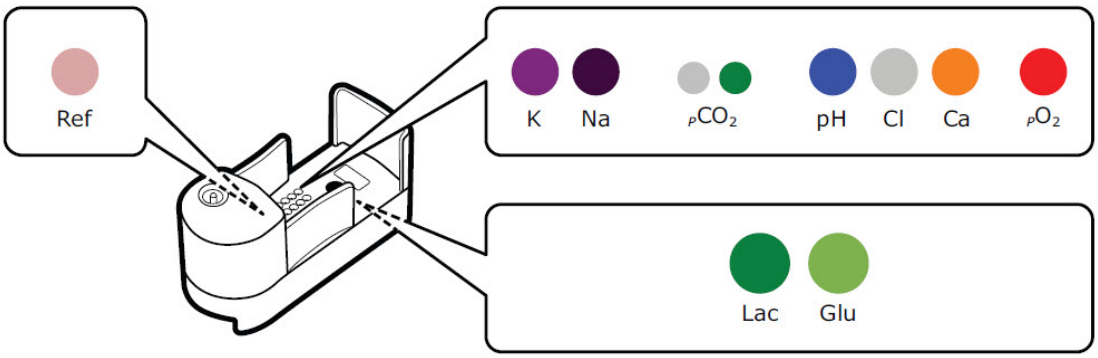

Figure 1: Sensor cassette diagram

The reference electrode maintains a stable, fixed potential against which the potentials of the pH and electrolyte electrodes can be measured. The sample composition has no effect on the potential of the reference electrode. The electrode maintains its potential due to the fact that the electrode is made of an Ag rod coated with AgCl in a solution with constant Cl^- concentration, which creates an Ag^+/Ag equilibrium.

1. Radiometer is a leading provider of technologically advanced acute care solutions that simplify and automate all phases of acute care testing

A diagram of the reference electrode is provided below (see Figure 2).

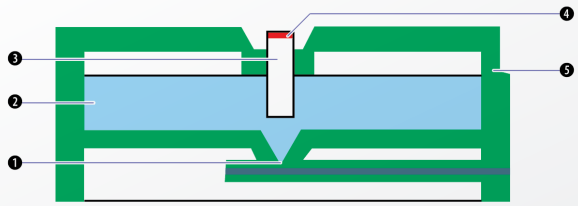

Figure 2: Reference electrode diagram

1. **Membrane** – Interface to the sample
2. **Electrolyte solution** – Acts as a salt-bridge solution that maintains an electrical contact between the electrode and the sample
3. **Electrode** – Provides the contact between the electrolyte and the electrical contact
4. **Electrical contact** – The point of electrical contact between the electrode and the analyzer

The analyzer utilizes a variety of measurement methods to measure the parameters of interest:

Potentiometry: Measures electrical potential. Used for pH, pCO_2, and electrolyte measurements

Amperometry: Measures the magnitude of an electrical current. Used when evaluating Glucose and Lactate concentrations

Optical pO_2 sensor: Used to detect pO_2

Spectrophotometry: Measures the absorption spectrum for parameters related to whole blood

The pH, electrolyte, and pCO_2 sensors are all of solid state design with a PVC (polyvinyl chloride) membrane, with the exception of the Cl^- sensor which has an epoxy membrane. The sensors utilize potentiometry by measuring the electric potential, or voltage, between the solid state contact of the sensor and the reference electrode. The potentiometric sensors all have slightly different designs, but the concepts are the same.

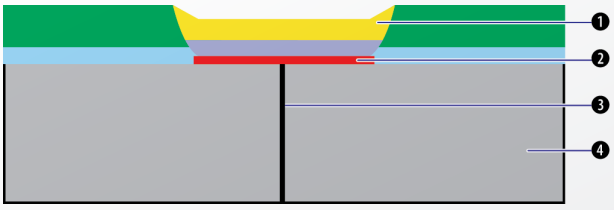

Figure 3: Potentiometric sensor diagram

1. **Membrane** – Ion-selective membrane that is in direct contact with the sample or calibration solution and that is sensitive to a certain ion, e.g. the H^+ ions
2. **Solid-state contact** - the point of electrical and ionic contact with the membrane
3. **Electrical contact** – The point of electrical contact between the sensor and the analyzer
4. **Sensor base** – The structural platform on which the sensor is formed

Amperometry is used to measure the glucose and lactate concentrations. In principle, the metabolite sensors are measuring the differences in potential along an electrode chain, and relating it to the concentration of the metabolite that is either being oxidized or reduced at the anode or cathode in the electrode chain.

A constant polarization voltage is applied to the electrode chain, and the current is measured by an amperemeter. As the glucose and lactate molecules are introduced to the sensor, they are converted to gluconic acid and pyruvate, respectively, along with H_2O_2. When a potential is applied to the electrode chain, the oxidation of H_2O_2 produces an electrical current proportional to the amount of H_2O_2, which is directly related to the amount of glucose/lactate.

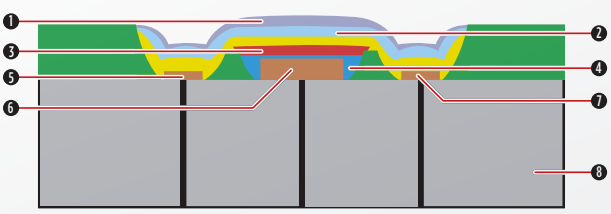

Figure 4: Glucose and Lactate sensor diagram

1. **Biocompatible layer** – Biocompatible layer
2. **Outer membrane** - Outer membrane permeable to glucose/lactate - diffusion control
3. **Enzyme layer** – Contains glucose/lactate oxidase
4. **Inner membrane** – Cellulose acetate
5. **Reference** – Ag/AgCl electrode
6. **Anode** – Platinum electrode
7. **Cathode** – Platinum electrode
8. **Sensor base** – The structural platform on which the sensor is formed

The pO_2 sensor is significantly different from the other sensors, since it uses an optical system. Its measurement principle is based on the ability of oxygen to reduce the intensity and time constant of the phosphorescence from a phosphorescent dye that comes in contact with the sample

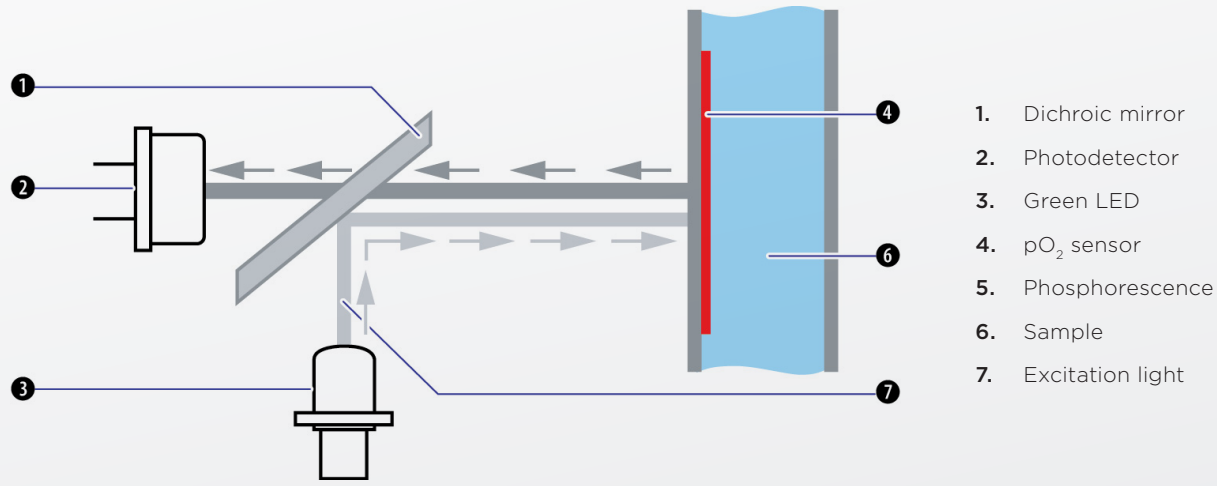

Figure 5: pO_2 sensor diagram

The light emitted by the green LED is reflected by a dichroic mirror onto the pO_2 sensor. Red light is emitted back through the dichroic mirror and onto a photodetector. The photodectector then sends the electrical signals, which are proportional to the intensity of the light, to the analog/digital converter and the data processing unit that calculates the pO_2 concentration

MetaFLEX RESULT FORMAT

The analyzer provides the results in a readable format on-screen and/or printed. Data logs are stored on-board for samples, quality control, and calibration results, as well as the activities of the analyzer. Results and data logs may also be exported.When exported from the analyzer, the data log files are in a .csv format, which allows for versatility when it comes to what types of programs can read the files.

RELIABILITY, ACCURACY, AND PRECISION

As mentioned previously, the Qualicheck 5+ series solutions can be used to perform external quality control on the MetaFLEX. These solutions can be used to test the accuracy and precision of the system. The Qualicheck solutions are contained in 2mL glass ampoules and have expected values and acceptable ranges for each of the MetaFLEX parameters. The expected values and ranges can be found on the data sheet provided with the solutions. It is important to use a new Qualicheck ampoule for each external run, as exposing the solution to air can compromise the pO_2, pCO_2 and pH values.

To demonstrate the performance and high correlation between instruments, results are provided from two Vi-CELL MetaFLEX units analyzing Qualicheck5+ levels 1-3. A total of three runs for each level were conducted. A fresh ampoule was used for each measurement. The values for each parameter from each level were averaged and were determined to have either passed or failed based on whether the average result fell within the expected range of values. The coefficients of variation (CV) for each parameter were tabulated in order to demonstrate the precision of the results.

Qualicheck5+ Testing Results on Unit 1:

Table 1. Qualichek Level 1 data - Unit 1

Qualicheck Level 1 - Lot 144								Expiration Date: 09-2017
Parameters		Mid Range	Min	Max	Result	Average Result	Std Dev	%CV
pH		7.07	7.05	7.09	**Pass**	7.07	0.001	0.01%
pCO_2	mmHg	71.0	66.0	76.0	**Pass**	67.90	0.100	0.15%
pO_2	mmHg	152	142	162	**Pass**	157.67	1.528	0.97%
cK^+	mmol/L	1.8	1.5	2.1	**Pass**	1.70	0.000	0.00%
cNa^+	mmol/L	162	158	166	**Pass**	162.00	0.000	0.00%
cCa^{2+}	mmol/L	1.00	0.90	1.10	**Pass**	0.98	0.000	0.00%
cCl^-	mmol/L	121	115	127	**Pass**	123.00	0.000	0.00%
cGlucose	g/L	0.26	0.18	0.36	**Pass**	0.29	0.006	2.01%
cLactate	g/L	0.38	0.32	0.43	**Pass**	0.40	0.000	0.00%

Table 2. Qualicheck Level 2 data - Unit 1

Qualicheck Level 2 - Lot 147								Expiration Date: 09-2017
Parameters		Mid Range	Min	Max	Result	Average Result	Std Dev	%CV
pH		7.395	7.375	7.415	**Pass**	7.39	0.001	0.01%
pCO_2	mmHg	40.9	37.9	43.9	**Pass**	40.53	0.252	0.62%
pO_2	mmHg	101	93	109	**Pass**	106.33	0.577	0.54%
cK^+	mmol/L	3.7	3.4	4	**Pass**	3.70	0.000	0.00%
cNa^+	mmol/L	142	138	146	**Pass**	141.00	0.000	0.00%
cCa^{2+}	mmol/L	0.53	0.43	0.63	**Pass**	0.52	0.000	0.00%
cCl^-	mmol/L	96	90	102	**Pass**	98.00	0.000	0.00%
cGlucose	g/L	1.02	0.88	1.17	**Pass**	1.01	0.006	0.57%
cLactate	g/L	0.15	0.10	0.19	**Pass**	0.15	0.006	3.94%

Table 3. Qualicheck Level 3 data - Unit 1

Qualicheck Level 3 - Lot 143								Expiration Date: 01-2018
Parameters		Mid Range	Min	Max	Result	Average Result	Std Dev	%CV
pH		7.583	7.558	7.608	**Pass**	7.58	0.001	0.01%
pCO_2	mmHg	22.6	20.1	25.1	**Pass**	22.20	0.000	0.00%
pO_2	mmHg	54.6	44.6	64.6	**Pass**	55.40	0.954	1.72%
cK^+	mmol/L	5.5	5.2	5.8	**Pass**	5.40	0.000	0.00%
cNa^+	mmol/L	127	123	131	**Pass**	127.00	0.000	0.00%
cCa^{2+}	mmol/L	0.37	0.27	0.47	**Pass**	0.37	0.006	1.55%
cCl^-	mmol/L	65	59	71	**Pass**	64.00	0.000	0.00%
cGlucose	g/L	2.40	2.07	2.72	**Pass**	2.45	0.006	0.24%
cLactate	g/L	0.93	0.82	1.05	**Pass**	0.97	0.006	0.60%

Qualicheck 5+ Testing Results on Unit 2:

Table 4. Qualicheck Level 1 data - Unit 2

Qualicheck Level 1 - Lot 144								Expiration Date: 04-2018
Parameters		Mid Range	Min	Max	Result	Average Result	Std Dev	%CV
pH		7.067	7.047	7.087	**Pass**	7.07	0.002	0.03%
pCO_2	mmHg	71.3	66.3	76.3	**Pass**	68.00	0.557	0.82%
pO_2	mmHg	154	144	164	**Pass**	154.67	0.577	0.37%
cK^+	mmol/L	1.5	1.2	1.8	**Pass**	1.70	0.000	0.00%
cNa^+	mmol/L	162	158	166	**Pass**	161.67	0.577	0.36%
cCa^{2+}	mmol/L	0.99	0.89	1.09	**Pass**	0.97	0.000	0.00%
cCl^-	mmol/L	122	116	128	**Pass**	122.33	0.577	0.47%
cGlucose	g/L	0.27	0.18	0.36	**Pass**	0.28	0.000	0.00%
cLactate	g/L	0.38	0.29	0.43	**Pass**	0.39	0.006	1.47%

Table 5. Qualicheck Level 2 data - Unit 2

Qualicheck Level 2 - Lot 147								Expiration Date: 02-2018
Parameters		Mid Range	Min	Max	Result	Average Result	Std Dev	%CV
pH		7.401	7.381	7.421	**Pass**	7.40	0.001	0.01%
pCO_2	mmHg	40.5	37.5	43.5	**Pass**	40.27	0.208	0.52%
pO_2	mmHg	100	92	108	**Pass**	100.33	0.577	0.58%
cK^+	mmol/L	3.8	3.5	4.1	**Pass**	3.70	0.000	0.00%
cNa^+	mmol/L	141	137	145	**Pass**	141.00	0.000	0.00%
cCa^{2+}	mmol/L	0.53	0.43	0.63	**Pass**	0.52	0.000	0.00%
cCl^-	mmol/L	97	91	103	**Pass**	98.00	0.000	0.00%
cGlucose	g/L	1.01	0.86	1.15	**Pass**	1.00	0.006	0.58%
cLactate	g/L	0.14	0.10	0.19	**Pass**	0.15	0.006	3.94%

Table 6. Qualicheck Level 3 data - Unit 2

Qualicheck Level 3 - Lot 143								Expiration Date: 01-2018
Parameters		Mid Range	Min	Max	Result	Average Result	Std Dev	%CV
pH		7.581	7.556	7.606	**Pass**	7.58	0.001	0.01%
pCO_2	mmHg	22.7	20.2	25.2	**Pass**	22.70	0.173	0.76%
pO_2	mmHg	55.7	45.7	65.7	**Pass**	54.13	0.306	0.56%
cK^+	mmol/L	5.5	5.2	5.8	**Pass**	5.40	0.000	0.00%
cNa^+	mmol/L	127	123	131	**Pass**	127.00	0.000	0.00%
cCa^{2+}	mmol/L	0.37	0.27	0.47	**Pass**	0.37	0.000	0.00%
cCl^-	mmol/L	65	59	71	**Pass**	65.67	0.577	0.88%
cGlucose	g/L	2.45	2.13	2.77	**Pass**	2.42	0.000	0.00%
cLactate	g/L	0.94	0.83	1.05	**Pass**	0.96	0.006	0.60%

Presented below are plots of the averaged results for pH, pCO_2 , pO_2, glucose, and lactate from both MetaFLEX instruments (see Figures 7-11).

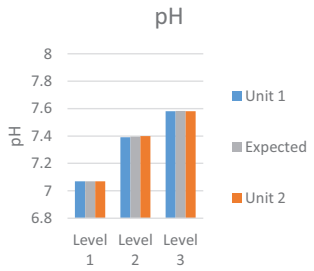

Figure 7: Comparison of Qualicheck5⁺ pH data

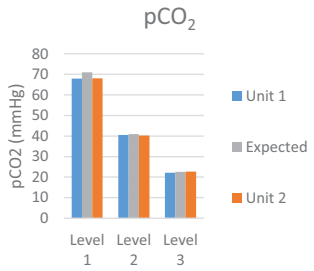

Figure 8: Comparison of Qualicheck5⁺ pCO_2 data

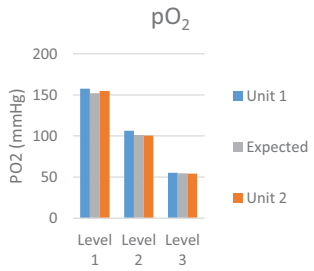

Figure 9: Comparsion of Qualicheck5⁺ pO_2 data

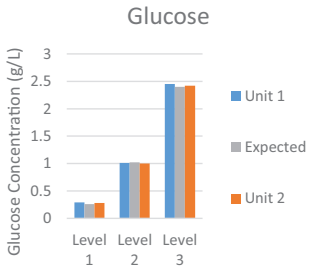

Figure 10: Comparison of Qualicheck5⁺ Glucose data

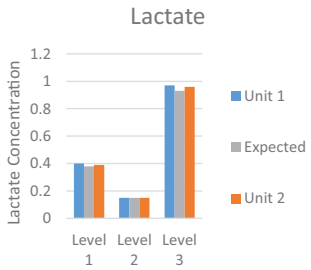

Figure 11: Comparison of Qualicheck5⁺ Lactate data

The low CV values for each parameter on both units combined with the high correlation results between the two units demonstrates the consistency and the precision of the Vi-CELL MetaFLEX.

In addition to testing the Qualicheck solutions, gravimetric solutions of glucose and lactate in DMEM media were tested. The glucose solutions were measured on two Vi-CELL MetaFLEX instruments (Units A & B) while the lactate solutions were only measured on one instrument (Unit C). The percent difference between the readings were calculated and the data was fit to a line of best approximation in order to determine the linearity and consistency of the results. The results are below.

Table 7. Glucose linearity data

Glucose					
Measured g/L	Unit A	Unit B	Average	Average CV%	% Difference
1.0	0.96	0.96	0.96	2.93	0.00
2.0	1.91	1.91	1.91	1.25	0.00
4.0	3.94	3.95	3.94	0.25	0.25
8.0	7.18	7.24	7.21	2.76	0.74

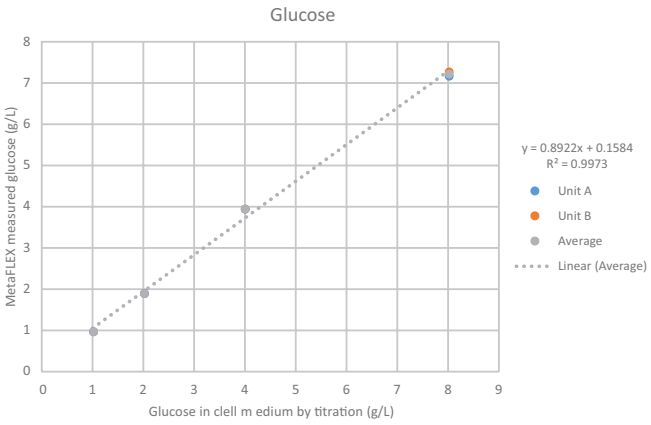

Figure 12: Glucose linearity data with line of best fit

Table 8. Lactate linearity data

Lactate					
Measured g/L	Average Reading	CV%	% Difference	Average CV%	% Difference
0.5	0.535	1.08	6.54	2.93	0.00
0.7	0.705	0.82	0.71	1.25	0.00
1.4	1.34	1.06	4.48	0.25	0.25
2.0	1.908	0.68	4.82	2.76	0.74

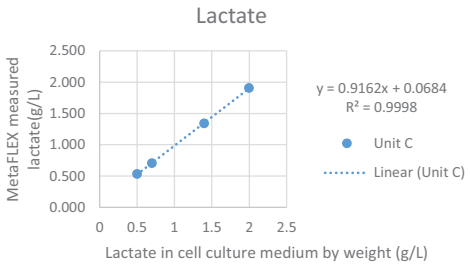

Figure 13: Lactate linearity data with line of best fit

According to the data, the MetaFLEX instruments were able to to provide linear responses to the changing concentrations of glucose and lactate with slopes of 0.89 and 0.92 and R^2 values of 0.99 for both parameters, respectively. The two instruments were also able to provide readings that resulted in average CV values under 3% and percent difference values under 10%.

RESULTS WITH CELL MEDIA

In order to demonstrate the usefulness of the Vi-Cell MetaFLEX testing was done on cell media from Green Fluorescent Protein (GFP) positive Chinese Hamster Ovary (CHO) cells. Cells were grown in media and samples of the media were taken each day over a ten day period. The samples were run on the Vi-CELL MetaFLEX to see the changes in the media composition over time. Special attention was given to glucose concentration, lactate concentration, and pH.

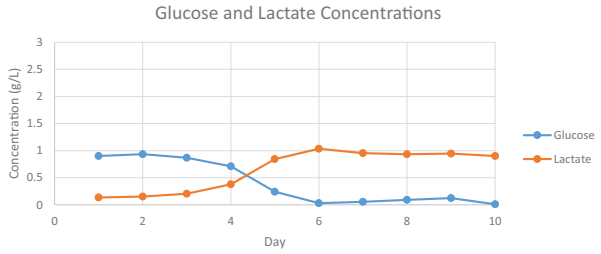

Figure 14: Glucose and Lactate concentrations in GFP CHO cells over time

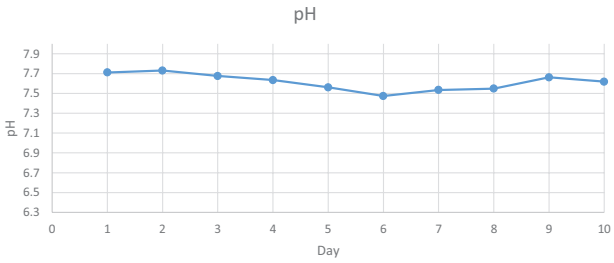

Figure 15: pH in GFP CHO cells over time

According to these charts, the glucose concentration decreased with time, while the lactate concentration increased with time, meaning both parameters changed as expected. The trends in pH are less evident as the expected decrease is small. These results do demonstrate the capability of the Vi-CELL MetaFLEX to analyze cell culture media.

Conclusion

The Vi-CELL MetaFLEX is an automated bioanalyte analyzer is an effective tool for analyzing mammalian and insect cell culture media. It measures the parameters most important in cell culture: pH, Glucose, Lactate, pCO_2, pO_2, and electrolytes. Ideal for small to large scale cell cultures as the MetaFLEX analyzer requires only a 65µL sample and provides results in 35 seconds. Samples can be introduced to the system via syringe, capillary tube, test tube, sample cup, and similar containers

References

Vi-CELL MetaFLEX Instructions for Use. 2016. Beckman Coulter Inc. Indianapolis, IN.

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