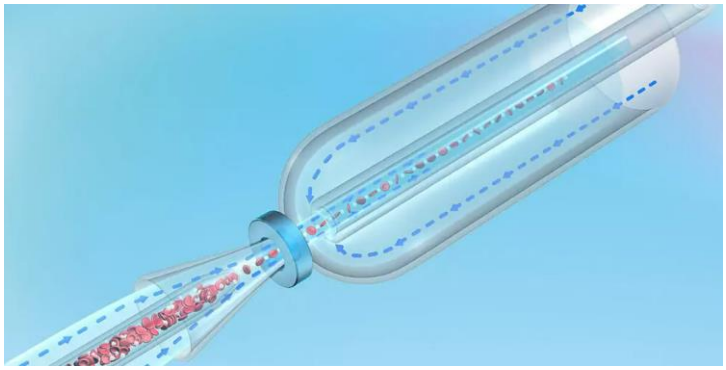
Appendix 2
Uses and substitutions of PFAS for Flow Cytometry Analyzer(IVD)

1. Fluorescence flow cytometry (FFC)

Fluorescence flow cytometry (FFC) is used to analyze physiological and chemical properties of cells. It provides:

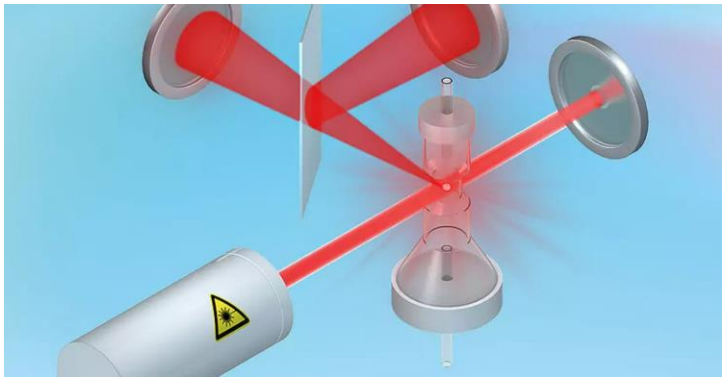
- Information about cell size and structure
- Information about a cell's interior

In flow cytometry, cells and particles are examined while they are flowing through a very narrow flow cell.

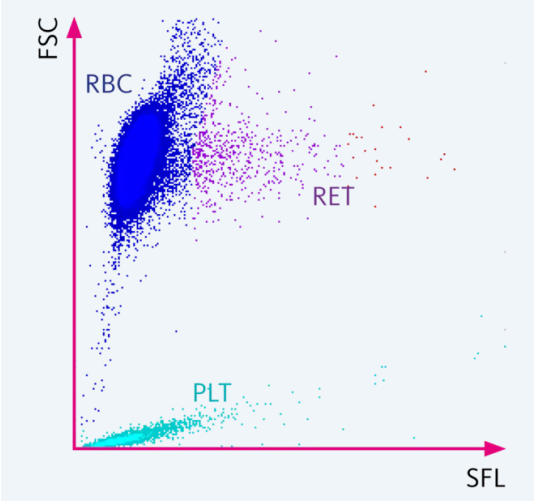


First a sample is aspirated and proportioned, then diluted to a pre-set ratio and labelled with a proprietary fluorescence marker that binds specifically to nucleic acids.

Next the sample is transported into the flow cell. The sample is illuminated by a semiconductor laser beam, and some different signals are detected.



Cells with similar physical and chemical properties form a cluster in a graph known as a scattergram.



The principle of fluorescence flow cytometry is used in different analyzers for haematology and urinalysis. In urinalysis analyzers, fluorescence technology is also used for counting bacteria, red blood cells, white blood cells and other elements.

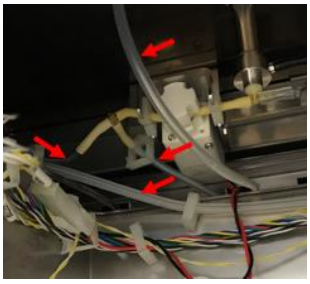
Furthermore, when a fluorescence flow cytometry analyzer is used in a medical setting, it is certified as a diagnostic device and is treated as an in vitro diagnostic device (IVD). On the other hand, when used in a laboratory, it is treated as a RUO (Research Use Only) device. These devices are the same, and the only difference is whether or not they have been certified as diagnostic devices.

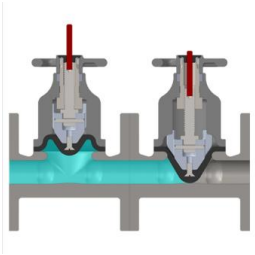
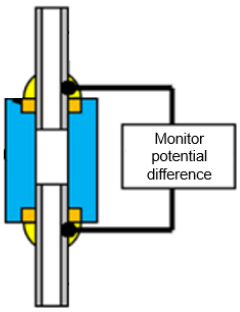
Figure1. IVD device	Figure2. RUO device
	

2. PFAS parts used in Fluorescence flow cytometry

In the chemical flow of fluorescence flow cytometry, strongly alkaline hypochlorous acid and organic solvents such as methanol, ethanol, and ethylene glycol flow. In addition, various specimens flow. Therefore, the parts that come in contact with reagents and specimens are basically required to have high chemical resistance. For example, joint parts between parts, O-rings, etc.

However, the most important parts are the parts that are essential for producing accurate measurement results, so we will focus on these important parts here.

Usage number	PFAS parts	Parts overview
1	Chemical tube (fluororesin) 	➢ Utilizing hardness, it is used for parts related to quantitativeness ➢ Comes into contact with various reagents (strongly alkaline hypochlorous acid, organic solvents such as methanol, ethanol, ethylene glycol, etc.) and specimens.
2	Double layer tube (Inner layer is fluororesin, outer layer is polyurethane)	➢ Utilizing flexibility, it is used where there is bending or where long distances are connected. ➢ Comes into contact with various reagents (strongly alkaline hypochlorous acid, organic solvents such as methanol, ethanol, ethylene glycol, etc.) and specimens.
3	Fluorine coated pipette 	➢ Used for dispensing reagents and inhaling specimens. ➢ Relates to quantitative nature. ➢ Comes into contact with various reagents (strongly alkaline hypochlorous acid, organic solvents such as methanol, ethanol, ethylene glycol, etc.) and specimens.
4	Diaphragm valve for chemical liquid	➢ Valves to control the flow of chemical solutions. ➢ The rubber membrane that comes into contact with the liquid inside the tube is

	 Left image: Fully open Right figure: Fully closed	PFAS. ➢ Comes into contact with various reagents (strongly alkaline hypochlorous acid, organic solvents such as methanol, ethanol, ethylene glycol, etc.) and specimens.
5	Sample presence sensor 	➢ A sensor that detects whether the sample remains on the stirring rod. ➢ The material of the sensor body (blue part in the left diagram) is PFAS. ➢ Comes into contact with various reagents (strongly alkaline hypochlorous acid, organic solvents such as methanol, ethanol, ethylene glycol, etc.) and specimens.

3. Substitutability verification results for each part using PFAS

No.1: Chemical tube (fluororesin)

(1) Required PFAS properties	chemical resistance, water repellency, quantitative properties (high hardness and little volume change), low reactivity to reagents
(2) Specifications required in the final product	➢ Chemical resistance to various reagents and samples (strongly alkaline hypochlorous acid, etc.) ➢ Ensuring quantification of specimens and reagents (suppression of variation in quantitative values, suppression of liquid droplets generated at the ejection port) ➢ Suppression of sample carryover ➢ Suppression of reagent carryover ➢ Low reactivity to reagents (material with high purity, little elution, high non-stickiness and little adhesion of impurities)
(3) Candidates for alternative materials that can meet the required	Nothing

specifications	
(4) Impact on the product when there is no alternative material	➢ Durability decreases, failures increase, and waste increases. ➢ The accuracy of measurement results decreases, and the quality of diagnosis and R&D deteriorates accordingly.
(5) Necessity of Exemptions under PFAS Restrictions	Exemption for more than 20 years is necessary since there are no other materials that can achieve (1) and (2).

No.2: Double layer tube (Inner layer is fluororesin, outer layer is polyurethane)

(1) Required PFAS properties	Chemical resistance, water repellency, low reactivity to reagents
(2) Specifications required in the final product	➢ Chemical resistance to various reagents and samples (strongly alkaline hypochlorous acid, etc.) ➢ Low reactivity to reagents (substance with high purity, little elution, high non-stickiness and little adhesion of impurities) ➢ Flexibility to fit compactly into equipment
(3) Candidates for alternative materials that can meet the required specification	Nothing
(4) Impact on the product when there is no alternative material	➢ Durability decreases, failures increase, and waste increases. ➢ If flexibility decreases, equipment becomes larger, transportation load increases, and installation locations are limited.
(5) Necessity of Exemptions under PFAS Restrictions	Exemption for more than 20 years is necessary since there are no other materials that can achieve (1) and (2).

No.3: Fluorine coated pipette

(1) Required PFAS properties	Chemical resistance, water repellency
(2) Specifications required in the final product	➢ Ensuring quantitative quality of specimens and reagents (suppressing variation in quantitative values) ➢ Suppression of carryover of liquid adhering to the pipette surface (If the pipette is used for dispensing reagents, the washing liquid will be brought into the reagent container, diluting the reagent and impairing the stability of the reagent.) ➢ Suppression of carryover between samples (cross contamination)
(3) Candidates for alternative materials that can meet the required specification	Nothing
(4) Impact on the product when there is no alternative material	➢ The accuracy of measurement results decreases, and the quality of diagnosis and R&D deteriorates accordingly.
(5) Necessity of Exemptions under PFAS Restrictions	Exemption for more than 20 years is necessary since there are no other materials that can achieve (1) and (2).

No.4: Diaphragm valve for chemical liquid

(1) Required PFAS properties	Chemical resistance
(2) Specifications required in the final	➢ Chemical resistance to various reagents (strong alkaline hypochlorous acid, organic solvents such as methanol, ethanol, ethylene glycol, etc.)

product	➢ Accurate control of chemical flow rate
(3) Candidates for alternative materials that can meet the required specification	Nothing
(4) Impact on the product when there is no alternative material	➢ Durability decreases, failures increase, and waste increases. ➢ The accuracy of measurement results decreases, and the quality of diagnosis and R&D deteriorates accordingly.
(5) Necessity of Exemptions under PFAS Restrictions	Exemption for more than 20 years is necessary since there are no other materials that can achieve (1) and (2).

No.5: Sample presence sensor

(1) Required PFAS properties	Non-conducting, chemical resistance, water repellency
(2) Specifications required in the final product	➢ Suppression of sample carryover ➢ Improved sensor detection accuracy by improving liquid drainage (water repellency)
(3) Candidates for alternative materials that can meet the required specification	Nothing (Evaluated with PVC, but there were many false detections due to liquid residue)
(4) Impact on the product when there is no alternative	➢ The accuracy of measurement results decreases, and the quality of diagnosis and R&D deteriorates accordingly. ➢ In the case of materials with low water repellency, detection accuracy decreases because liquid remains.

material	
(5) Necessity of Exemptions under PFAS Restrictions	Exemption for more than 20 years is necessary since there are no other materials that can achieve (1) and (2).