

Determination of poly- and perfluoroalkyl substances (PFAS) in blood by liquid chromatography tandem mass spectrometry

Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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Table of Contents

1. Introduction	3
2. Methodology.....	4
2.1. Technical Parameters for PFAS Analysis in Venous Serum and Capillary Whole Blood	4
2.2. Blood specimens	5
2.3. Standards and Quality Controls	5
2.4. Sample preparation.....	5
2.5. Instrumental method	6
2.6. Method validation.....	6
2.7. Quality Controls	11
3. Proficiency tests	11
Annex	28

1. Introduction

This report describes the analytical methodology used for the quantitative determination of poly- and perfluoroalkyl substances (PFAS) in blood by liquid chromatography tandem mass spectrometry (LC-MS/MS) for the following targeted substances:

- perfluorobutanoic acid (PFBA)
- perfluoropentanoic acid (PFPeA)
- perfluorohexanoic acid (PFHxA)
- perfluoroheptanoic acid (PFHpA)
- perfluorooctanoic acid– linear (PFOA) and branched (PFOA-B)
- perfluorononanoic acid (PFNA)
- perfluorodecanoic acid (PFDA)
- perfluorundecanoic acid (PFUnDA)
- perfluorododecanoic acid (PFDoA or PFDoDA)
- perfluorobutane sulphonic acid (PFBS)
- Perfluorohexane sulphonic acid – linear (PFHxS) and branched (PFHxS-B)
- perfluoroheptane sulphonic acid (PFHpS)
- perfluorooctane sulfonic acid – linear (PFOS) and branched (PFOS-B)

In short, serum samples were analyzed by spiking an extraction internal standard into an aliquot of the serum sample prior to sample extraction, followed by protein precipitation with methanol.

For the analysis of capillary venous blood, the volumetric absorptive microsampling devices were extracted with an 80% methanolic solution in the presence of internal standard solution. After mixing and centrifugation, the supernatant and methanolic sample solution respectively, was analyzed.

Calibration standards and internal quality controls (QC's) were prepared by using the working solutions in blank matrix. In each analytical batch, calibrators, quality controls, a system and extraction blank were analyzed.

Extracts from samples, calibrators and controls were analyzed applying ultra performance liquid chromatography mass spectrometry. Detection was performed using multiple reaction monitoring mode. The calibration curves were constructed by plotting the peak area ratios between each analyte and internal standard versus the concentration to obtain a quantitative result for PFAS targeted substances.

2. Methodology

PFAS compounds were measured in blood serum and capillary venous blood (fingerprick). Most perfluorinated compounds occur only in their linear form; some, such as PFOS, PFOA, and PFHxS, contain mixtures of both linear and branched isomers, which are found in nature and in humans.

The following PFAS compounds were analysed in human serum and capillary venous blood.

- Perfluorobutanoic acid (PFBA)
- Perfluoropentanoic acid (PFPeA)
- Perfluorohexanoic acid (PFHxA)
- Perfluoroheptanoic acid (PFHpA)
- Perfluorooctanoic acid (PFOA) – linear and the sum of linear and branched forms
- Perfluorononanoic acid (PFNA)
- Perfluorodecanoic acid (PFDA)
- Perfluoroundecanoic acid (PFUnDA)
- Perfluorododecanoic acid (PFDoDA)
- Perfluorobutanesulfonic acid (PFBS)
- Perfluorohexanesulfonic acid (PFHxS) – linear and the sum of linear and branched forms
- Perfluoroheptanesulfonic acid (PFHpS)
- Perfluorooctanesulfonic acid (PFOS) – linear and the sum of linear and branched forms

2.1. Technical Parameters for PFAS Analysis in Venous Serum and Capillary Whole Blood

The method was validated with reference to ICH M10 on bioanalytical method validation scientific guideline.

The following technical parameters can be applied for the determination of PFAS in serum and capillary blood:

- Limit of Detection (LOD) and Limit of Quantification (LOQ): 0.1 µg/L
- Linearity
 - o Without dilution: 0.1 – 500 µg/L
 - o Dilution included: 0.1 – 2500 µg/L
- Measurement uncertainty: ≤ 30% (for linear forms)

2.2. Blood specimens

Blood samples were collected by ISSeP and transported for analysis to the laboratory of Eurofins. On arrival in the lab, the blood collection tubes (BD Vacutainer SST II Advance Plus Blood Collection Tubes) were centrifuged (1000g; 10 min) and an aliquot of the homogenized serum was kept in a PFAS free container at -20°C until analysis.

Capillary venous blood sampled using volumetric absorptive microsamplers (Mitra®volumetric microsampling (VAMS) device - Neoteryx Corporation, Torrance, CA, USA) were collected by ISSeP and transported for analysis to the laboratory of Eurofins. Sampling devices were kept in closed packages with desiccant at room temperature until analysis

2.3. Standards and Quality Controls

Certified multicomponent stock solutions (2000 ng/ml) for the calibrators and isotope labelled analogues applied as extraction internal standard were obtained from Wellington Laboratories.

The stock solution for the extraction internal standard contained, among others, the following isotope labelled analogues: 13C4 PFBA; 13C5 PFPeA; 13C5 PFHxA; 13C4 PFHpA; 13C8 PFOA; 13C9 PFNA; 13C6 PFDA; 13C7 PFUnDA; 13C3 PFBS; 13C3 PFHxS and 13C8 PFOS.

The certified multicomponent stock solution used for the preparation of the internal QC was purchased from AccuStandard and matrix matched QC's (AM-S-Y2301 and AM-S-Y2302) were purchased from Centre de Toxicologie du Québec. Calibration standards (n=8, working range: 0.1 - 500 ng/ml serum) and QC Mid (5.0 ng/ml serum), were prepared in blank matrix (Fetal Bovine Serum) by using dilutions of the stock solutions.

2.4. Sample preparation

- Serum

To a 50 µl aliquot of serum, extraction internal standard was spiked. After homogenization, protein precipitation with 200 µl methanol was performed. After centrifugation, the supernatant was injected in the liquid chromatograph with mass spectrometer.

- Capillary venous blood

An extraction was performed onto the tip of the volumetric absorptive microsampling devices with an 80% methanolic solution and addition of internal standard. After mixing and centrifugation, the methanolic sample solution was injected in the liquid chromatograph with mass spectrometer.

2.5. Instrumental method

The LC-MS/MS analysis was performed on an Acquity UPLC system (Milford, MA, WATERS, USA) coupled to an Absolute triple quadrupole mass spectrometer (Milford, MA, WATERS, USA) equipped with PFAS solution installation kits (Milford, MA, WATERS, USA).

Sample separation was performed using on a WATERS Acquity UPLC UPLC HSS T3 1,8 μm , 2,1 mm x 100 mm fitted with a 1.8 μm HSS T₃ column using a gradient elution program with mobile phases 2 mM ammoniumacetate in water and 2 mM ammoniumacetate in methanol respectively, with column temperature set at 40 °C. The temperature of the autosampler was set at 10 °C and the injected volume was 5 μl .

The mass spectrometer was equipped with an electrospray interface operating in negative ionization mode. The source temperature and desolvation temperature were set at 120 °C and 500 °C, respectively. The desolvation gas flow was set at 1000 liter/hour.

Detection was performed using multiple reaction monitoring (MRM) with two transitions for each analyte (except for PFBA and PFPeA for which only one transition is available) and one transition for the internal standards. When two transitions are available, the first transition was used for quantification and the second for qualification.

The calibration curves were constructed by plotting the peak area ratios between each analyte and internal standard versus the concentration to obtain a quantitative result for PFAS targeted substances. For branched compounds PFOA-B, PFHxS-B and PFOS-B, a result was obtained based on the calibration curves of their respective linear compounds.

2.6. Method validation

Both methods—for serum and capillary blood analysis—were validated. Validation parameters and performance criteria were established based on the ICH M10 guideline for bioanalytical method validation.

For quantifying branched isomers (branched PFOS, PFOA, and PFHxS), calibration was performed using their respective linear forms. A specific PFAS compound may contain various branched isomers. Currently, there is no universally standardized protocol for quantifying branched forms, so quantification based on the corresponding linear form is common practice. Due to method-specific parameters that affect detection of branched isomers, this can lead to method-specific differences between laboratories for branched PFAS components.

Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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Measurement uncertainty indicates how accurate a measurement is and the range within which the “true” result likely falls. This is expressed as a 95% confidence interval, meaning there is 95% certainty that the actual value lies within that range. For linear components, a measurement uncertainty of <30% was required. As previously mentioned, there is no standardized protocol for quantifying branched isomers (branched PFOS, PFOA, and PFHxS), so no specific uncertainty requirements can be set for these components.

Table 1. Summary of Validation Results for targeted PFAS in matrix human serum and venous blood

Parameter	Acceptance criteria	Human serum	Venous blood
Calibration model	range: 0,1 – 500 ng/mL linear model preferred (8 calibrator levels (cal L); 0.10 ng/mL; 0.20 ng/mL; 0.50 ng/mL; 1.00 ng/mL; 10.0 ng/mL; 100 ng/mL; 250 ng/mL and 500 ng/mL) * Back-calculated concentrations of each calibration standard should be within $\pm 20\%$ of the nominal concentration at the LLOQ and within $\pm 15\%$ at all the other levels. * At least 75% of the calibration standards should meet the above criteria. - 75% of all calibrators and at least 50% of all calibrators within each level	For all PFAS linear model with $1/x^2$ weighting ⁽¹⁾	For all PFAS linear model with $1/x^2$ weighting ⁽¹⁾
Limit of detection / lowest limit of quantification	* LLOD and LLOQ were defined as the value of the lowest non-zero calibrator. * LLOQ meet requirements of bias and precision.	0.1 ng/mL	0.1 ng/mL

Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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Parameter	Acceptance criteria	Human serum	Venous blood
Bias and precision (within and between run)	The maximum acceptable bias shall be $\pm 15\%$; with maximum acceptable bias at concentrations level LLOQ: $\pm 20\%$ Determined on spiked QC's at concentration levels LLOQ (0.10 ng/ml); LOW (0.25 ng/ml), MID (5.0 ng/ml) and HIGH (400 ng/ml) for linear compounds.	Within run precision: 1.3% - 18.8% Between run precision: 3.8% - 14.5% Bias: -11.1% - 19.0%	Within run precision: 3.7% - 13.4% Between run precision: 0.0% - 9.5% Bias: -15.8% - 7.2%
Internal standard recovery	Set to 50 – 200%	50 – 200%	50 – 200%
Selectivity / interference	Area < 20% of area LLOQ and area < 5% of area LLOQ for internal standard In case of presence of PFAS analyte: ion ratio < 30% deviation from obtained ion ratio in QC MID ⁽²⁾	No observed interference for all the compounds, with exception: * PFHpA: limited exceedance of ion ratio criterium in 1 human matrix * PFBA: in 1 human matrix, ion ratio criterium could not be assessed (one transition)	No observed interference for all the compounds, with exception: * PFBA: in 2 human matrices, ion ratio criterium could not be assessed (one transition) * PFPeA: in 2 human matrices (limited exceedance) * PFHxA: in 3 human matrices (in qualifier) * PFOA-B: in 1 human matrix * PFOS-B in 1 human matrix

Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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Parameter	Acceptance criteria	Human serum	Venous blood
Ionisation suppression / enhancement (matrix effect)	CV% of the internal standard compensated matrix effect must not exceed 15% Determined on spiked QC's at concentration levels LOW (0.25 ng/ml), MID (5.0 ng/ml) and HIGH (400 ng/ml) for linear compounds.	2.1% - 13.4%	3.3% - 13.2%
Carry-over	Area < 20% of area LLOQ and area < 5% of area LLOQ for internal standard	* Cal L6 onwards: PFHxS * Cal L7 onwards: PFHxA, PFOA, PFNA, PFDA, PFUnDA and PFDoA * Cal L8 onwards: PFHpA and PFHpS ⁽³⁾	* Cal L5 onwards: PFOA, PFNA, PFUnDA and PFOS * Cal L6 onwards: PFDoA * Cal L7 onwards: PFDA * Cal L8 onwards: PFHxS ⁽³⁾
Dilution integrity	Bias and within run precision must not exceed 15% for diluted sample (dilution factor 5)	All acceptance criteria are met for all compounds except PFBA and PFPeA.	All acceptance criteria are met for all compounds
Processed sample stability on autosampler	Bias must not exceed 15% relative to concentration at T0 Determined on spiked QC's at concentration levels LOW (0.25 ng/ml) and HIGH (400 ng/ml)	72 hours	72 hours

Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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Parameter	Acceptance criteria	Human serum	Venous blood
Stability storage conditions	Bias must not exceed 15% relative to concentration at T₀ Determined on spiked QC's at concentration levels LOW (0.25 ng/ml) and HIGH (400 ng/ml)	All acceptance criteria are met for all compounds for at least 3 weeks at room temperature (15-25°C) and at 4°C. All acceptance criteria are met for all compounds for at least 3 weeks at -20°C except for PFUnDA with limited bias exceedance for QC High	All acceptance criteria are met for all compounds for at least 2 weeks at room temperature (15-25°C) except for PFBA, PFBS, PFPeA and PFUnDA (for QC Low) and PFDoA (for QC High)
Freeze-thaw stability	Bias must not exceed 15% relative to concentration at T₀ Determined on spiked QC's at concentration levels LOW (0.25 ng/ml) and HIGH (400 ng/ml)	2 freeze-thaw cycles	2 freeze-thaw cycles

- ⁽¹⁾ Calibration model for PFDoA: linear model with 1/x² weighting applying PFUnDA isotope labelled analogue as internal standard
- ⁽²⁾ Blood samples from healthy adult volunteers were found to contain one or more PFAS compounds. Therefore, an ion ratio criterium was applied. In analysis batches, fetal bovine serum was therefore used as matrix blank, that only contains a small amount of PFOS and PFOS-B.
- ⁽³⁾ The technical procedure (SOP) describes how carry-over must be managed.

2.7. Quality Controls

Each analysis batch contains calibrators (0.10 ng/ml; 0.20 ng/ml; 0.50 ng/ml; 1.00 ng/ml; 10.0 ng/ml; 100 ng/ml; 250 ng/ml and 500 ng/ml serum), blank injections, system blank sample and double blank sample, QC samples (AM-S-Y2301, AM-S-Y2302 and MID), the latter analyzed in replicate before and after sample injections.

During the technical review and batch release were evaluated:

- residuals of calibration curve
- blank injections and system blank samples to verify for carry-over and contamination
- the measured PFAS quantities of MID QC are monitored and evaluated using a control chart for individual compounds
- the measured PFAS quantities of matrix controls AM-S-Y2301 en AM-S-Y2302 are evaluated against supplier specifications
- internal standard recovery

The QC measured values are used to monitor and evaluate the extended measurement uncertainty as a function of time.

3. Proficiency tests

The laboratory periodically participates in proficiency tests:

- Proficiency test: AMAP test for persistent organic pollutants in human serum (AMAP).
- Proficiency test organizer: Institut national de Santé Publique Québec, Centre de Toxicologie

The proficiency test reports are available upon request. The report of the most recent proficiency test AMAP 2025-2 is provided in Annex I as an example.

An overview of Eurofins proficiency test results for individual PFAS are shown in the format of a trend chart. The following alarming zones of z-scores are applied:

- If $-2 \leq z\text{-score} \leq 2$:

Then the assessment cell ("Beoordeling") is shown with **green** background and interpreted as "goed" (or 'satisfactory')

- If $-3 < z\text{-score} \leq -2$ or $2 \leq z\text{-score} < 3$

Then the assessment cell ("Beoordeling") is shown with **orange** background interpreted as "twijfelachtig" (or 'Need attention')

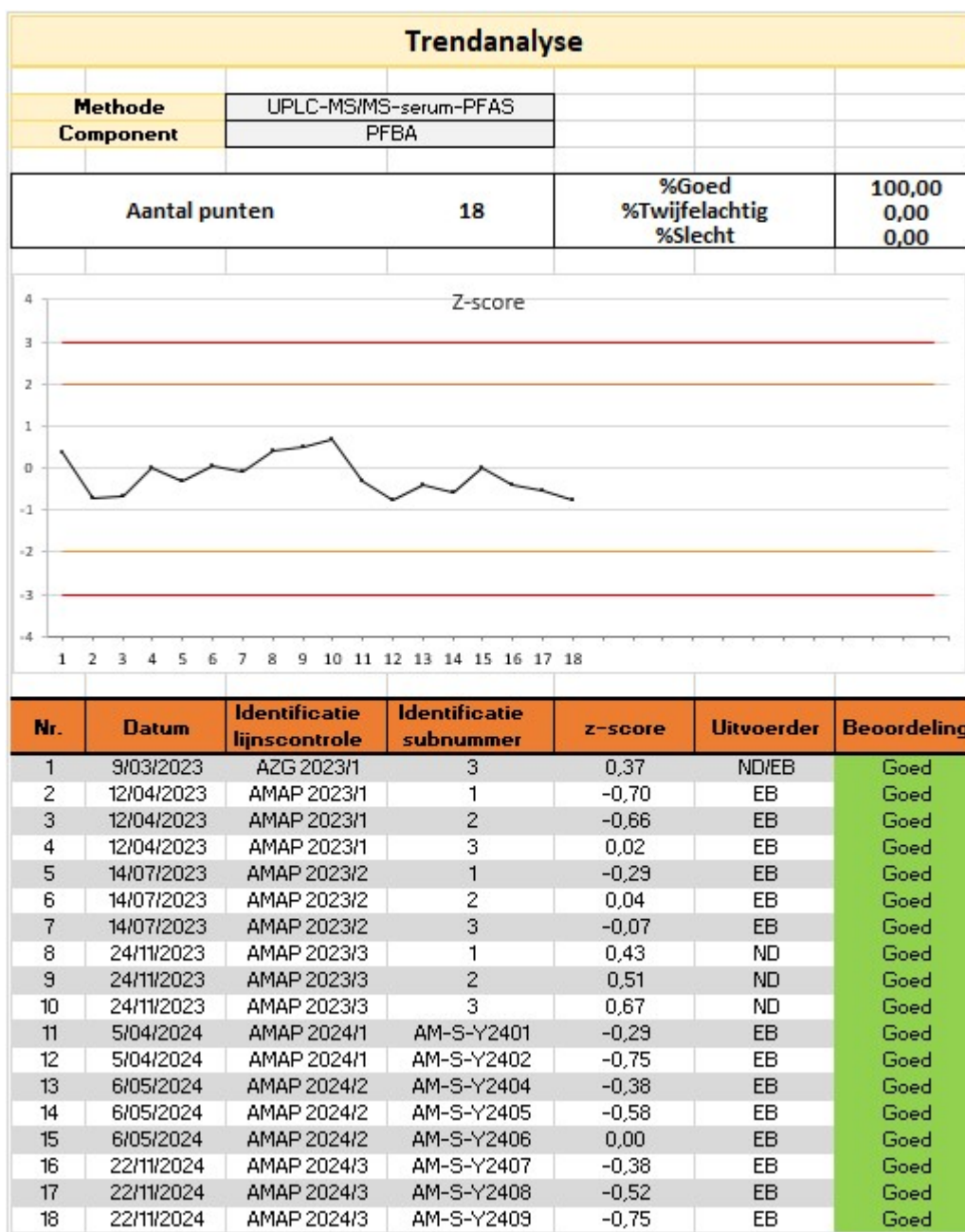
- If $z\text{-score} \leq -3$ or $z\text{-score} \geq 3$

Then the assessment cell ("Beoordeling") is shown with **red** background and interpreted as "slecht" (or 'Unsatisfactory')

Remarks:

- For proficiency test AMAP 2024/1 for substance PFDA, AMAP 2023/1 for branched-PFOS and AMAP 2025/1 for substance PFDoA a z-score of respectively -2.01, 2.17 and 2.09 was obtained.
For each, a deviation in the laboratory's quality system was registered and further investigated.
- Only if the respective PFAS was present in the proficiency test sample, an entry was made in the trend chart. Therefore, not all trend charts contain the same number of entries.
- For targeted PFAS not included in the proficiency tests, a second line check is performed by the laboratory.

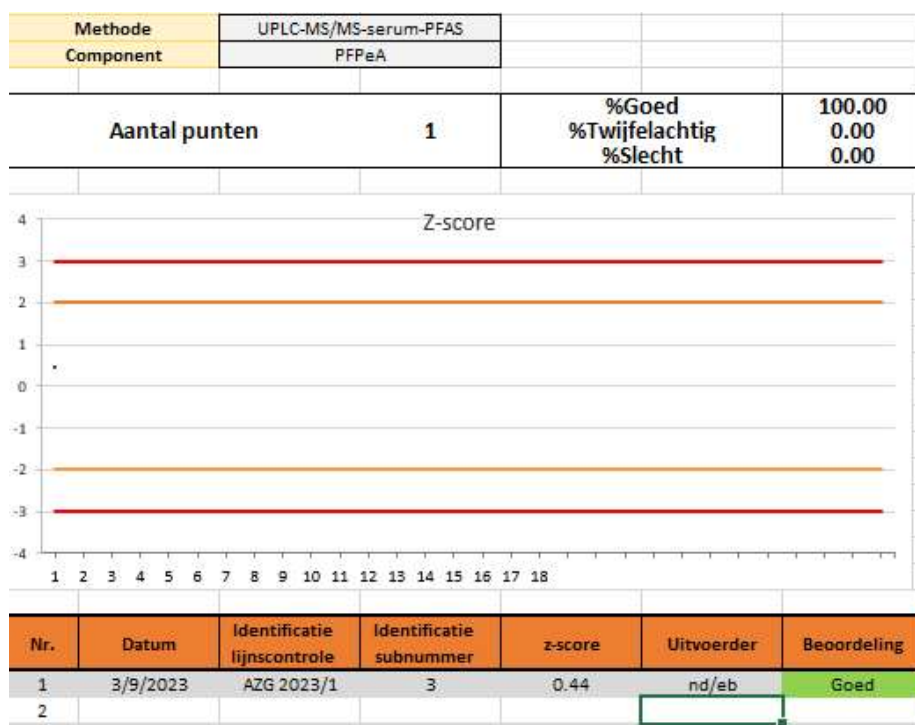
- Figure 1: Trend chart of perfluorobutanoic acid (PFBA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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- Figure 2: Trend chart of perfluoropentanoic acid (PFPeA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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- Figure 3: Trend chart of perfluorohexanoic acid (PFHxA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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- Figure 4: Trend chart of perfluoroheptanoic acid (PFHpA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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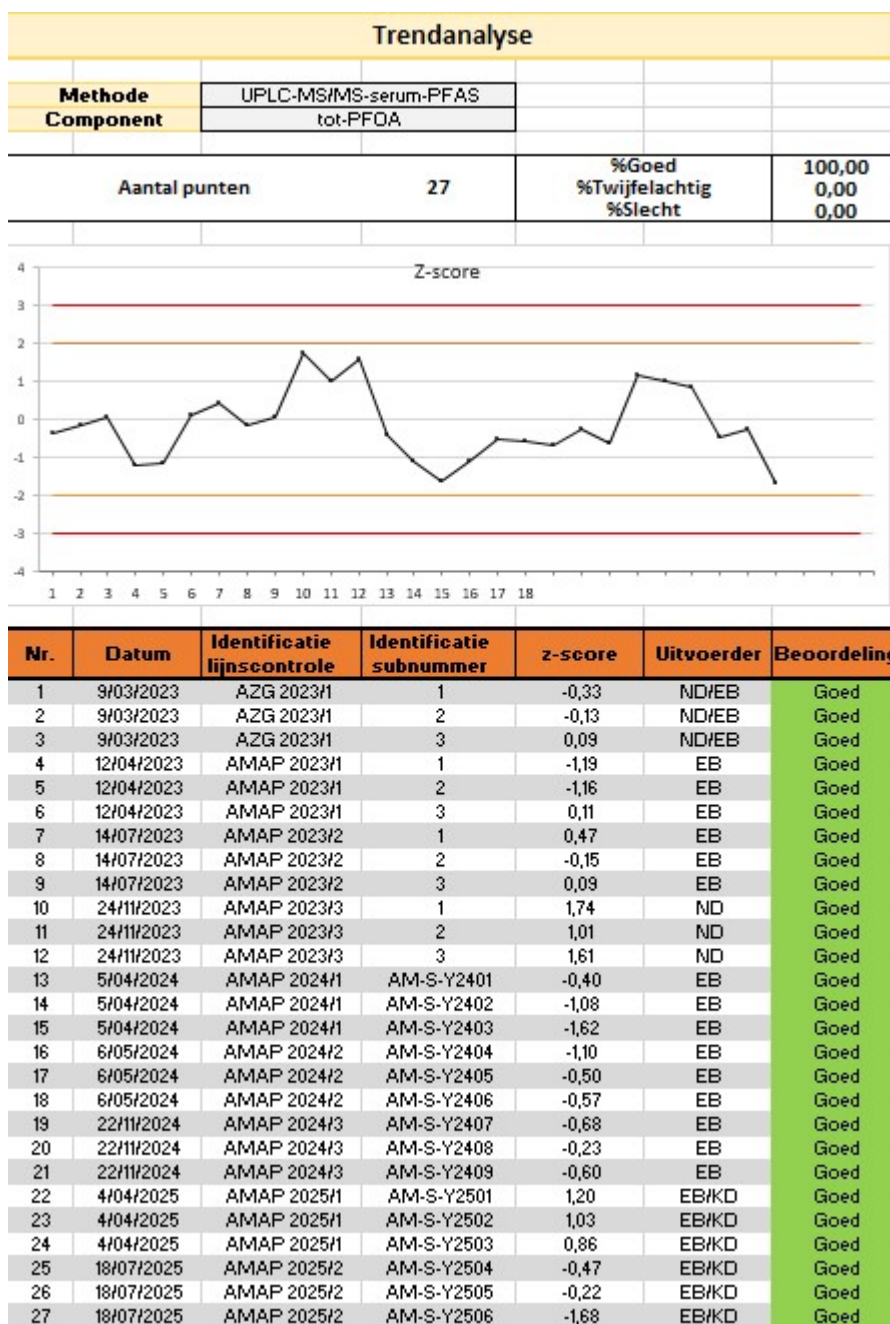
- Figure 5a: Trend chart of perfluorooctanoic acid linear (L-PFOA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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- Figure 5b: Trend chart of perfluorooctanoic acid total (tot-PFOA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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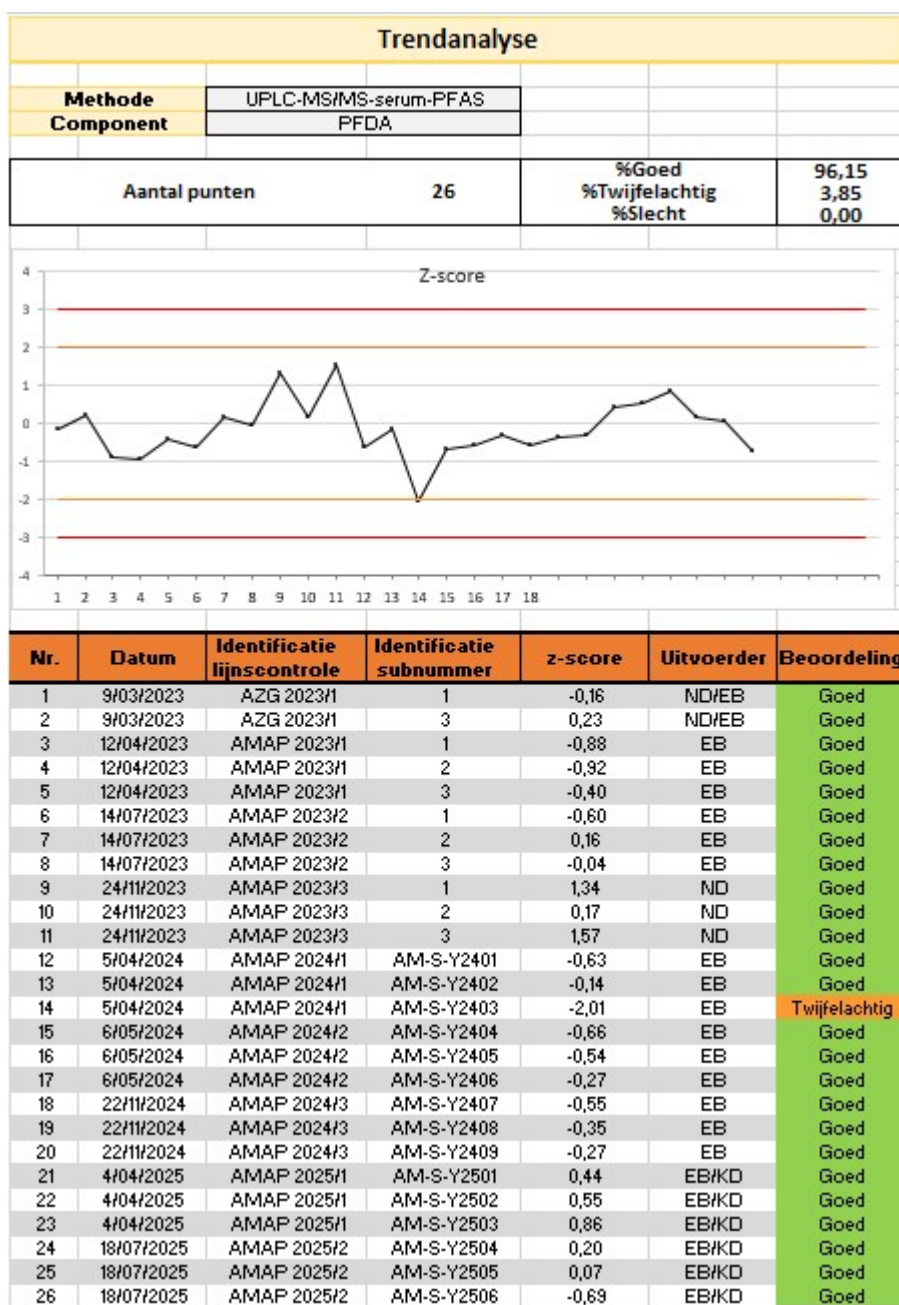
- Figure 6: Trend chart of perfluorononanoic acid (PFNA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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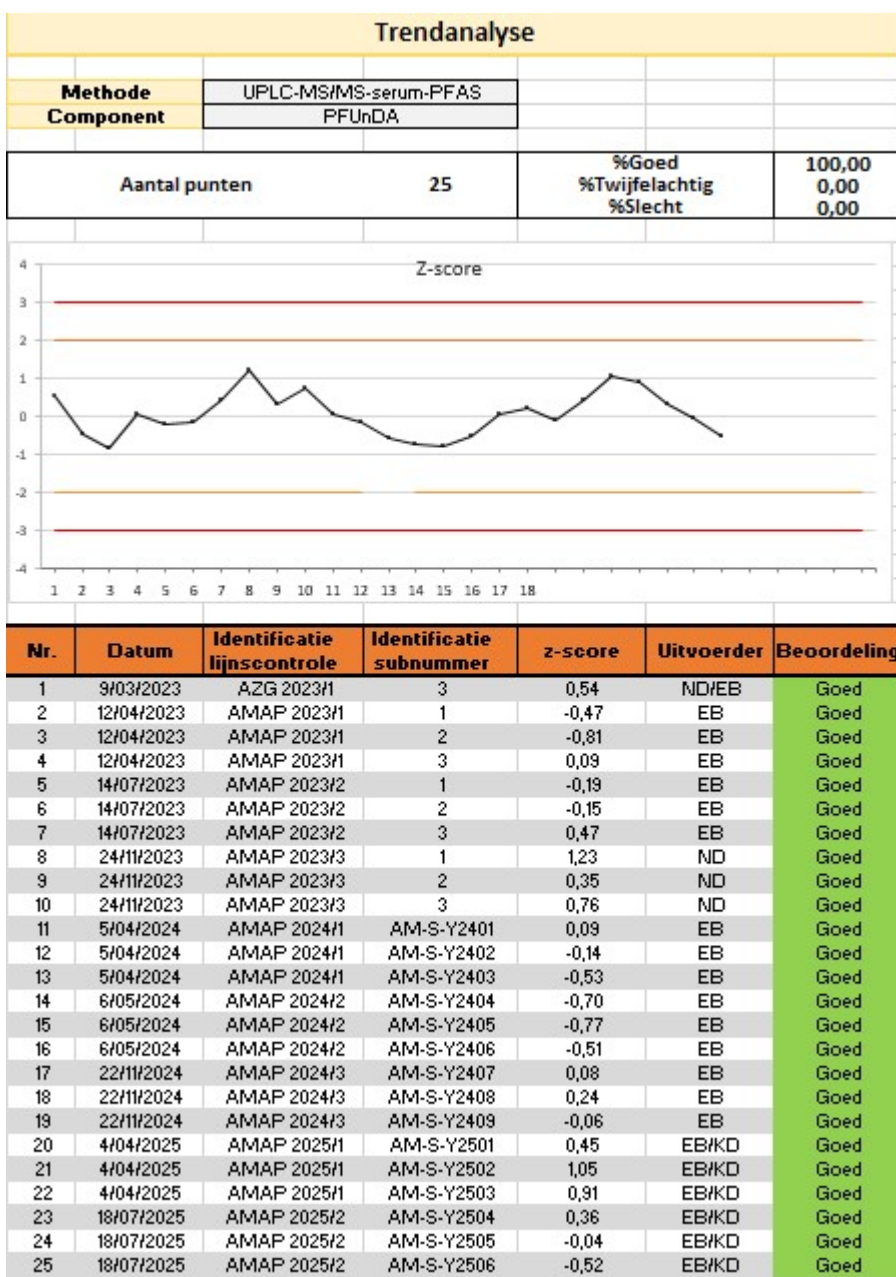
- Figure 7: Trend chart of perfluorodecanoic acid (PFDA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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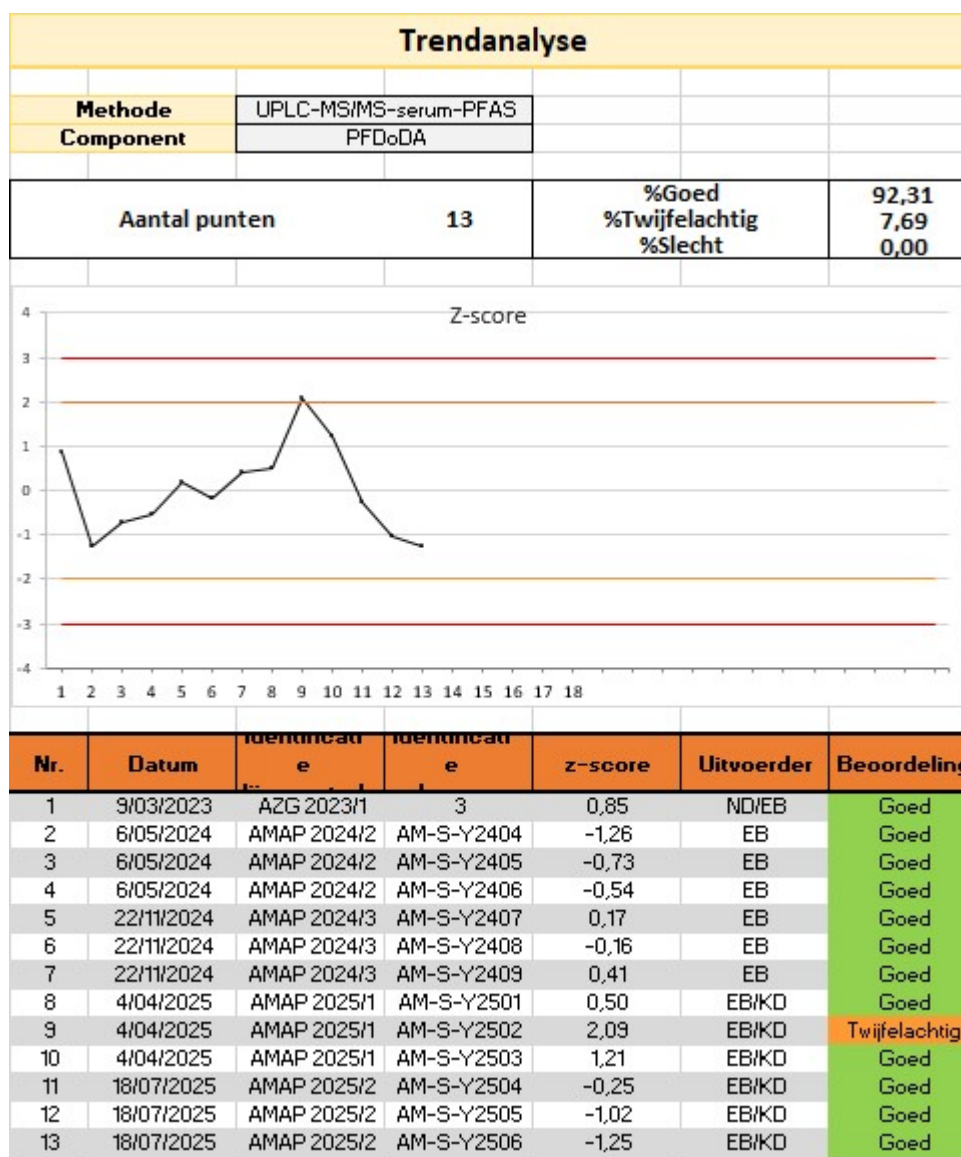
- Figure 8: Trend chart of perfluoroundecanoic acid (PFUnDA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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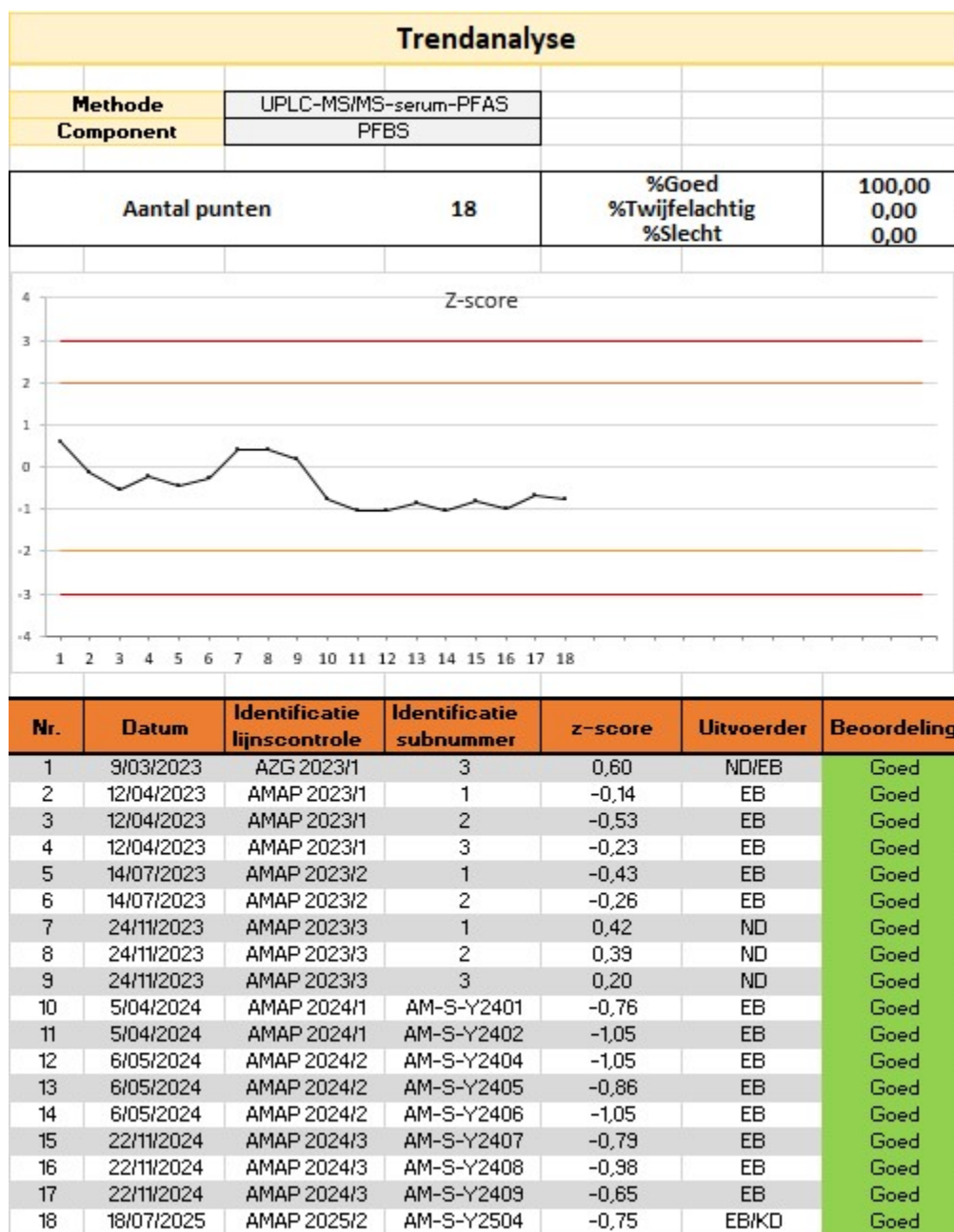
- Figure 9: Trend chart of perfluorododecanoic acid (PFDoA or PFDoDA) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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- Figure 10: Trend chart of perfluorobutane sulphonic acid (PFBS) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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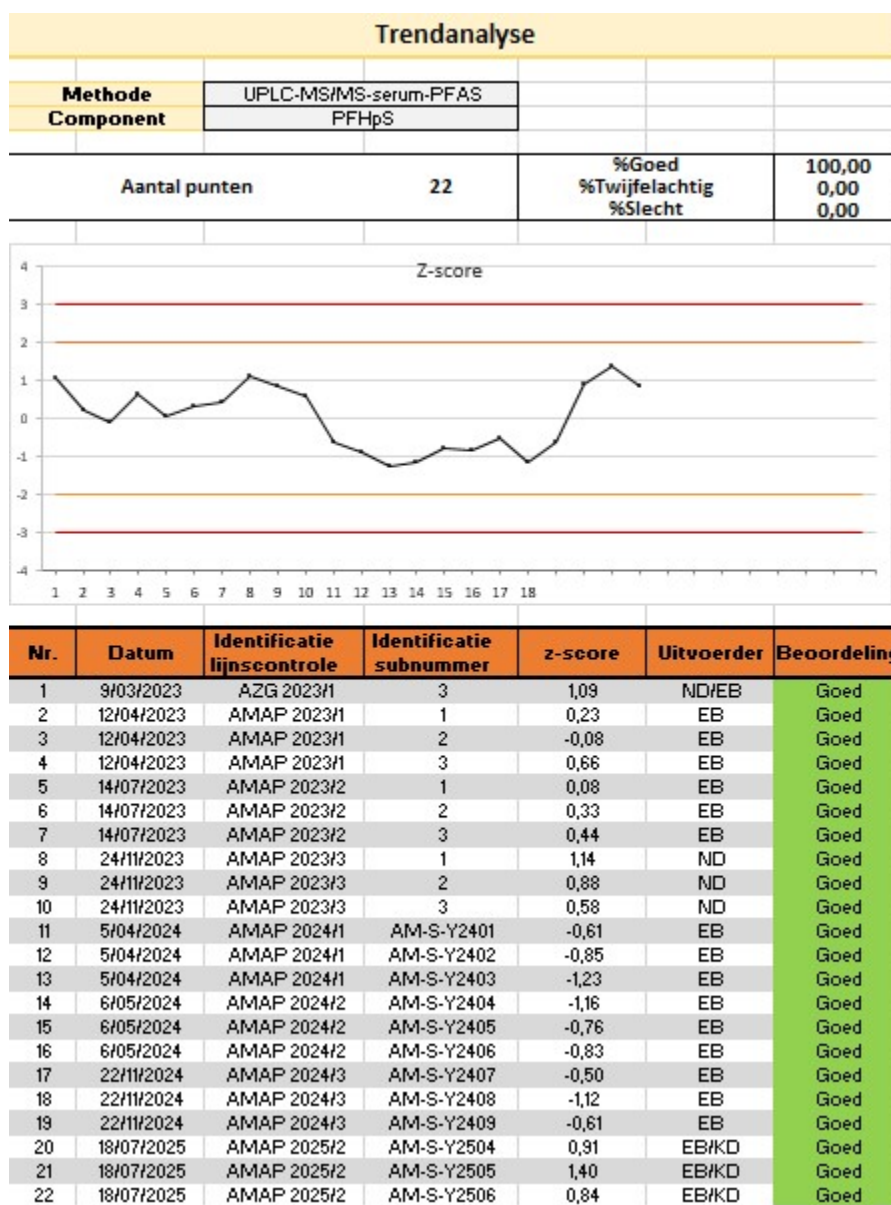
- Figure 11: Trend chart of perfluorhexane sulphonic acid total (tot-PFHxS) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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- Figure 12: Trend chart of perfluorheptane sulphonic acid (PFHpS) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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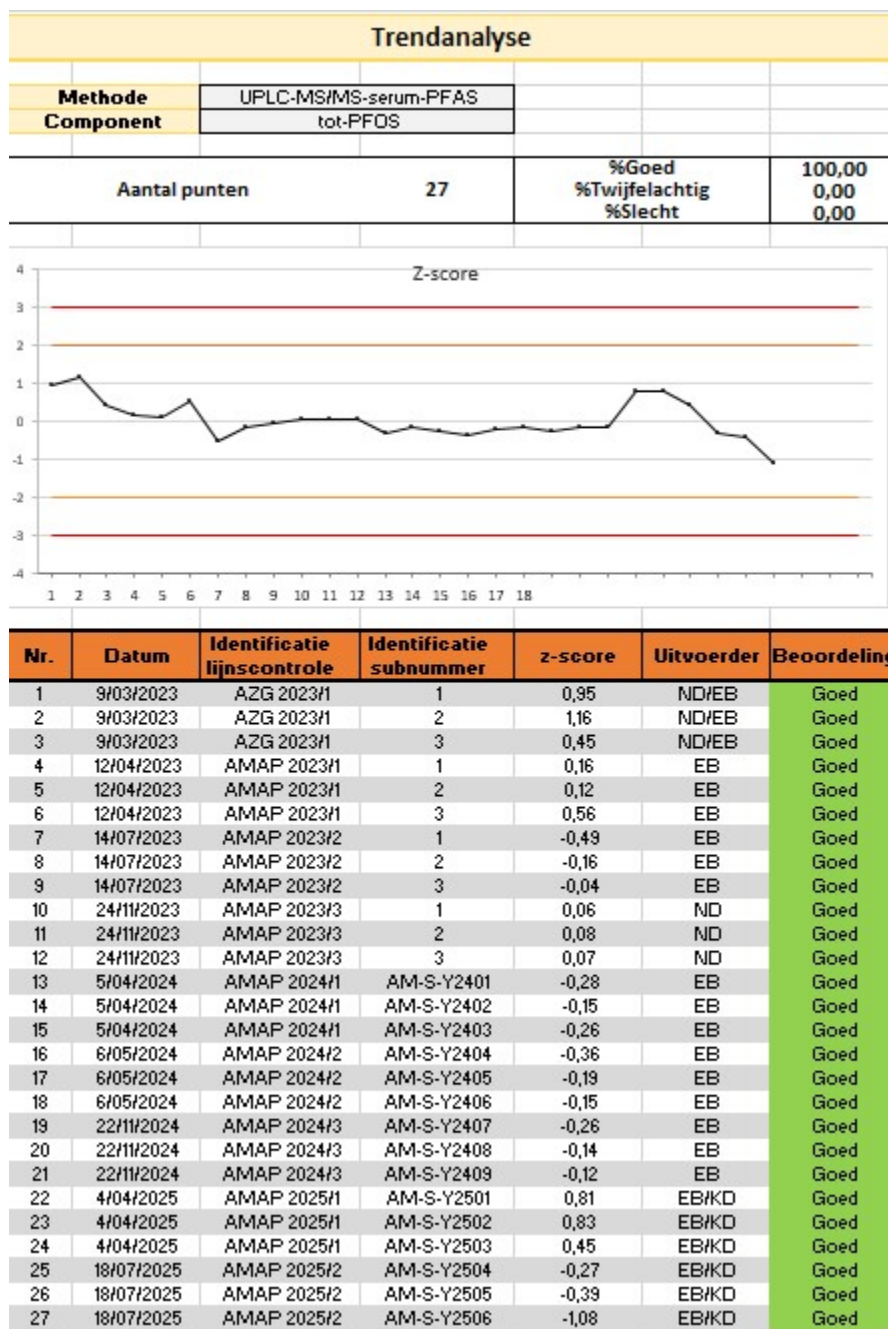
- Figure 13a: Trend chart of perfluorooctane sulphonic acid linear (L-PFOS) proficiency test results



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- Figure 13b: Trend chart of perfluorooctane sulphonic acid total (tot-PFOS) proficiency test results



Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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Annex

Annex I: report proficiency test AMAP 2025-2

Determination of PFAS in blood by liquid chromatography tandem mass spectrometry – September 2025

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