Development of a ready-to-use LC-MS kit for quantifying mAbs plasma levels in patients with cancer, and cross-validation of this multiplex solution

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SUMMARY

Objective: This work describes the evaluation of an original multiplex LC–MS/MS method using a ready to-use kit to simultaneously assay 7 mAbs in plasma.

Delivrables: CE-IVD ready-to-use kit allowing the monitoring of Nivolumab, Pembrolizumab, Ipilimumab, Atezolizumab, Bevacizumab, Rituximab, Cetuximab and Trastuzumab.

Technology: LC-MS/MS.

Measuring range: 2 µg/mL to 100 µg/mL concentration range, with no dilution required.

Samples validated: Plasma or serum

Analytical validation: It meets the EMA/FDA guidelines.

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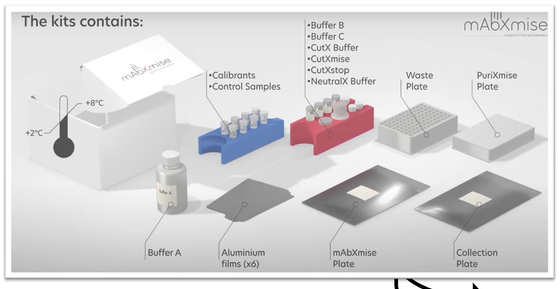
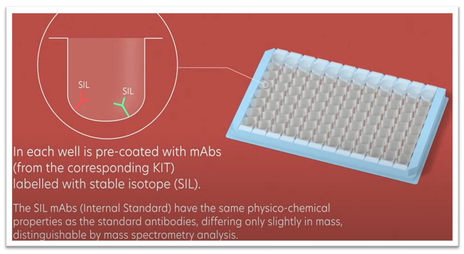
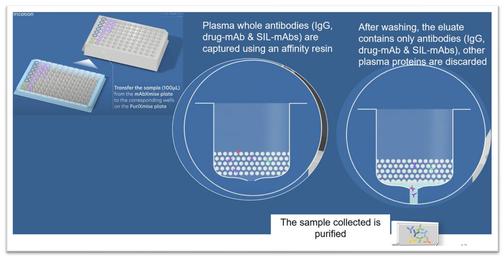
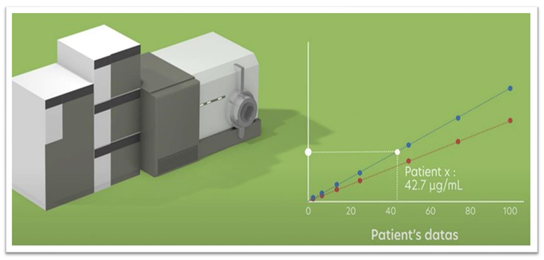
PRINCIPLE



The ONC-TDM-01 mAbXmise kit is a unique commercial solution for measuring C_{trough} concentrations in patient plasma and serum. It contains a stable-isotopically version of the 7 mAbs, ready-to-use calibrators and QC, and all the reagents and consumables required for sample preparation prior to LC-MS injection.







Ready-to-use CAL, QC and reagents:

► gain of time

► limits handling errors

mAbXmise plate:

► SIL-mAbs into mAbXmise plate wells

► Equal quantity in each well

► Resolubilized with patient plasma

► Overall process biases normalisation

PuriXmise plate:

► Whole IgG content, including t-mAbs and SIL-mAbs, are purified from the sample

► Decomplexified sample is then digested and injected onto the LC-MS/MS instrument.

Samples prepared with the kit can be analysed with several configurations of LC-MS/MS instruments, QqQ or HRMS.

PERFORMANCES

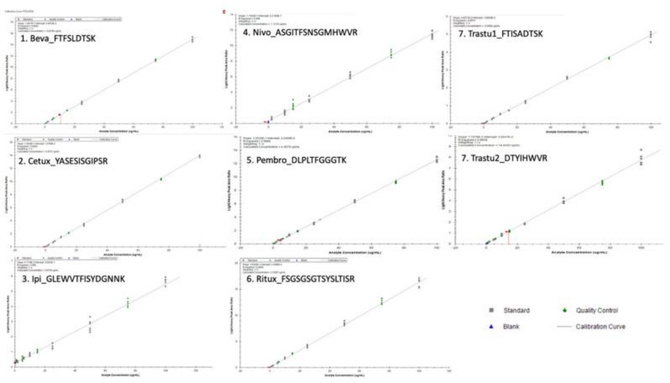


Figure 2. Slopes, intercepts and coefficient of determination mean values were respectively as follows (n = 6): y = 0.15X + 0.025 (r^2 = 0.999) for bevacizumab (Beva), y = 0.14X + 0.012 (r^2 = 0.999) for cetuximab (Cetux), y = 0.052X + 0.26 and (r^2 = 0.986) for Ipi, y = 0.11X + 0.32 (r^2 = 0.995) for Nivo, y = 0.13X + 0.032 (r^2 = 0.998) for pembrolizumab (Pembro), y = 0.16X + 0.044 (r^2 = 0.996) for rituximab (Ritux), y = 0.05X + 0.018 (r^2 = 0.997) for trastuzumab_pep1 (Trastu1) and y = 0.08X + 0.040 (r^2 = 0.994) for trastuzumab_pep2 (Trastu2) where x is the concentration in µg/mL and y is the area ratio. Selectivity 3 rry-over and matrix effect.

mAbXmise ONC-TDM-01 kit and LC-MS method were evaluated for analytical performances according to EMA/FDA guidelines. Data were acquired on a 6500 Qtrap coupled to an Exion chromatography. Separation of peptides was achieved using a Biozen™2.6µm Peptide XB-C18 LC column 100x2.1 mm (Phenomenex).

For all the mAbs, the LLOQ was 2 µg/mL and ULOQ was 100 µg/mL, which enables to cover a wide range of plasma concentrations. LLOQ is higher than concentrations previously reported with ELISA methods for the targeted mAbs. Inter-assay precision and accuracy for all mAbs ranged [1.0-13.1%] and [1.3-107.1%] respectively. Intra-assay precision and accuracy were < 14.6% and ranged from 90.1 to 111.1% respectively.

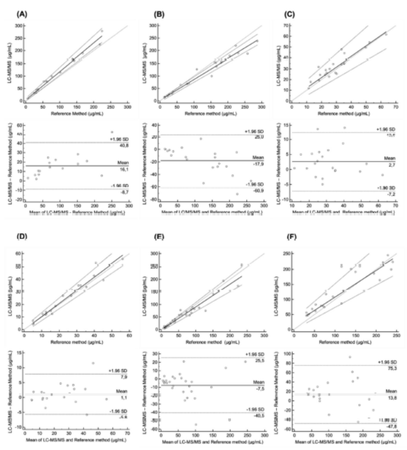
Nominal Concentration (µg/mL)	Level	Within-Run: Mean Calculated Concentration (µg/mL)	Within-Run: Intra-Day Precision [a] (%, n = 6)	Within-Run: Intra-Day Accuracy (%, n = 6)	Between-Run: Mean Calculated Concentration (µg/mL)	Between-Run: Inter-Day Precision [a] (%, D = 4, n = 16)	Between-Run: Inter-Day Accuracy (%, D = 4, n = 16)
Pembrolizumab DLPLTFGGGTK							
2	LLOQ	1.9	4.2	95.7	1.9	2.1	93.0
6	Low IQC	6.0	2.1	100.4	6.1	4.4	100.7
15	Mid IQC	14.9	1.4	99.3	15.4	2.5	102.3
75	High IQC	73.1	1.5	97.5	74.8	1.6	100.0
Rituximab FSGSGSGTSYSLTISR							
2	LLOQ	2.0	6.9	100.5	2.1	4.6	106.8
6	Low IQC	6.5	5.4	107.8	6.4	2.1	106.0
15	Mid IQC	16.1	3.8	107.5	16.1	1.8	107.1
75	High IQC	77.5	4.0	103.3	77.5	1.8	103.3

Example of data obtained for 2 mAbs: Pembrolizumab and Rituximab.

Selectivity, carry-over, matrix effect and dilution were assessed and found in good agreement with the EMA/FDA specifications.

Analysis for cross-validation were succefully obtained in different clinical labs either with a TSQ Altis (Thermo) or with a Xevo TQ-XS (Waters). Full data available in the following article: Manin *et al.*, **Pharmaceuticals**, 2021.

COMPARISON



Passing-Bablok and Bland-Altman plots. On top, Passing-Bablok regression plot of concentrations measured by LC-MS/MS and reference method for A) Bevacizumab; B) Cetuximab; C) Nivolumab; D) Pembrolizumab; E) Rituximab; F) Trastuzumab.
Bottom are displayed the Bland-Altman analysis of the difference between LC-MS/MS and reference method for the same mAbs.

- The mean absolute bias of concentrations between multiplex and reference methods was 9,82 % (range [3.0-11.1%]).
- Cusum test was not statistically significant for each mAb, thus confirming the linear relationships between the methods.
- Bland Altman analysis showed that mean estimated bias for each mAb was acceptable and has no incidence on the analytical results.
- Full data in the paper below

pharmaceuticals MDPI

Article

Cross-Validation of a Multiplex LC-MS/MS Method for Assaying mAbs Plasma Levels in Patients with Cancer: A GPCO-UNICANCER Study

Clémence Marin [1,2,3], Nihel Khoudour [4], Aurélien Millet [5], Dorothée Lebert [6], Pauline Bros [6], Fabienne Thomas [7,8], David Ternant [9,10], Bruno Lacarelle [1,2], Jérôme Guitton [5,11], Joseph Ciccolini [1,2,3] and Benoit Blanchet [4,12,*]

Conclusion and Take-Home messages

The key arguments for TDM of mAbs in oncology are :

• Early prediction of a patient's response or non-response to a treatment while limiting toxicity, thanks to information on under-dosing and over-exposure.

• A useful tool for improving progression free survival and overall survival.

• Management of economic toxicity.

Conclusion:

We have successfully developed a commercial CE-IVD kit for monitoring concentrations of 7 different mAbs used in oncology. The analytical performances are validated according to EMA guidelines. This kit is a robust and easy bioanalytical solution developed for being implemented in clinical lab by pharmacologists who are willing to propose TDM for mAbs. Our solution is already implemented in several hospitals in Europe.