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Total Value of Ownership and Overall Equipment Effectiveness analysis to evaluate the impact of automation on time and costs of therapeutic drug monitoring

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HIGHLIGHTS

- Quality indicators for evaluating therapeutic drug monitoring sector organization.
- Time and costs of analyses investigated by Total Value of Ownership.
- Overall Equipment Effectiveness to assess Availability, Performance and Quality.
- Automation coupled to LC-MS improves all novel indicators.
- Integrated analysis as useful tool to help activity planning and improvements.

GRAPHICAL ABSTRACT

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ABSTRACT

Total Value of Ownership (TVO) and Overall Equipment Effectiveness (OEE) analysis are novel tools capable of monitoring and analyzing industrial processes by assessing the efficiency of the entire instrumental equipment and calculating instrument capacity utilization. Such integrated analysis, measuring quality indicators of the testing process, could also provide new perspectives and methodologies for the workflow organization of clinical laboratories. In this study, TVO and OEE were employed for the evaluation of two different configurations of a therapeutic drug monitoring sector, comparing the results obtained for immunosuppressant (ISD) and anti-epileptic drugs (AED) analysis as well as checking their quantitative performance in terms of limit of quantification, accuracy and precision. TVO analysis was performed for ISDs, including the Total Direct Labor Time, Total Cycle Time and Turnaround Time as well as cost of testing. Instruments' performance and workload were assessed using OEE indicator, studying Availability, Performance and Quality factors. Total Cycle Time for a batch was 3.55 h, decreasing of 1.5 h in the new setting where personnel are engaged for 0.98 h, 25% of total testing time. The calculated cost per sample was 6.60 euro. Availability values were significantly higher for automated sample-handling system and ISDs analysis by LC-MS. Higher Performance values were obtained for LC-MS system for AED and other TDM. Quality values were >0.94 for all instruments. TVO and OEE proved to be applicable to clinical laboratory environment, quantifying benefits and costs of newly developed semi-automated therapeutic drug monitoring sector. This novel approach based on an integrated analysis may

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help activity planning and quality improvement and could be used in the future for benchmarking progress as a product/process comparison tool in other laboratory fields.

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List of abbreviations

AED	Anti-epileptic Drugs
ASPS	Automated Sample Preparation System
BRIV	Brivaracetam
CARB	Carbamazepine
CSA	Cyclosporin
D	Availability
DLT	Direct Labor Time
EVER	Everolimus
FENB	Phenobarbital
FENT	Phenytoin
GAB	Gabapentin
ISD	Immunosuppressant
LAC	Lacosamide
LAM	Lamotrigine
LEV	Levetiracetam
MHD	Oxcarbamazepin
MPA	Mycophenolic Acid
OEE	Overall Equipment Effectiveness
P	Performance
PREG	Pregabalin
Q	Quality
RET	Retigabine
RUF	Rufinamide
SIR	Sirolimus
STIR	Stiripentol
TACR	Tacrolimus
TAT	Turnaround Time
TDM	Therapeutic Drug Monitoring
TIAG	Tiagabinae
TOP	Topiramate
TVO	Total Value of Ownership
VALP	Valproic Acid

1. Introduction

The hub-and-spoke model promoted the centralization of highly specialized sectors such as Therapeutic Drug Monitoring (TDM). This prompted hub laboratories to introduce new technologies in order to face the workload increase. TDM concept evolved from the simple dosage of drugs in biological fluids to a helpful tool for the clinician in the individualized management of therapy [1–5]. The most commonly used analytical platforms for TDM analysis are immunoassays and liquid chromatography-tandem mass spectrometry (LC-MS) [6]. Although LC-MS represents the gold standard for xenobiotics' quantification in biological matrices for its specificity and sensitivity, many laboratories use immunoassays because they are automated and can be easily performed 24/7. Several studies compared these analytical approaches, showing an overestimation of results obtained by immunomediated techniques mainly due to lack of specificity [7–10]. Nevertheless, LC-MS is not free from drawbacks: interfering isobaric substances or high matrix effects may be observed, causing a reduction in sensitivity. Moreover, LC-MS is still not easy to automate, requires a high level of know-how and until a few years ago a limited number of validated diagnostic kits for in vitro diagnosis (IVD) were available on the market [11]. More recently, automated liquid-handling systems have been introduced for pre-analytical steps, facilitating reagent dispensing, temperature-controlled incubation, mixing and centrifugation even with complex matrices such as whole blood [12].

The increase in business volumes and the growing demand for rapid response from the laboratory in general and, to an even greater extent, in specialized sectors, such as TDM, are associated with an increase in investment for training and initial costs. To continue creating value while remaining in a context of economic sustainability, laboratories must aim to optimize their processes. To do this it is necessary to monitor and analyze the laboratory as a whole with new perspectives and methodologies; in this scenario, the measurement of the *Total Value of Ownership* (TVO) can offer new possibilities [13]. TVO is based on an accurate assessment of the overall costs of the analytical cycle in the context of the laboratory and provides an estimate of all direct and indirect costs associated with the acquisition of an analytical system for its entire life span. To improve and monitor analytical performance as well as workflow and overall organization of laboratories, numerous quality indexes have been developed [14], including indicators of validation process and others suitable to assess the total testing cycle. Significant indicators of testing cycle are *Turnaround Time* (TAT) [15], defined as the interval between a specimen's arrival in the laboratory and the time the result is issued, *Total Cycle Time*, deriving from the sum of all the times needed to complete the diverse analytical phases, and *Direct Labor Time* (DLT), based on the time spent by the laboratory staff in testing procedures, thus providing tools to detect slower or aberrant steps [16] and low-value and/or manual activities. An additional tool capable of assessing the efficiency of the entire instrumental equipment of the laboratory is the *Overall Equipment Effectiveness* (OEE) [17]. It is a performance indicator commonly used in industry to calculate instrument capacity utilization and it is based on three categories: Availability, Performance and Quality. Availability takes into account planned time loss and determines how strongly the capacity of the machine for the value-added functions related to the planned availability is; Performance represents the efficacy measure of a process and takes into account delays and speed loss; Quality rate is the relationship of the proper quantity to the produced quantity and takes into account part loss tracks.

The aim of this work was to apply different quality indicators for the evaluation of the new organization of our laboratory comparing the results of immunosuppressant (ISD) and anti-epileptic drugs (AED) with those obtained with the past configuration. In particular, TVO analysis was performed to calculate testing time data, including the total DLT, *Total Cycle Time* and TAT, as well as cost of testing. Furthermore, OEE indicator, commonly used in industry and based on actual instrument availability time, process performance and quality, was used to assess the efficiency of the entire

instrumental equipment of the laboratory. Although it is evident that this type of analysis is not strictly related to the development and validation of analytical methods, the presented approach could provide novel tools to analytical chemistry experts to monitor and analyze the performance of a laboratory configuration, taking into account also the cost of analysis which is often an important point to be considered when developing and implementing novel methodologies.

2. Materials and methods

2.1. Laboratory organization

Instrumental set-up for TDM until November 2017 consisted of two LC-MS and two HPLC-UV instruments. Four ISDs and twelve AEDs were measured with LC-based methods. TDM analyses were performed by immunoassays, for determination of CSA, five AEDs, three antibiotics and benzodiazepines. The new instrument configuration includes an automated sample preparation system (ASPS; Hamilton Microlab STAR™ let ivd, Hamilton Company, Reno, NV, USA) and three novel LC-MS platforms (Shimadzu Nexera X2 and Sciex Triple Quad 4500MD). The latter, being mirrored to each other, are used in rotation and as back-up to each other for all LC-MS assays. The analytes monitored are five ISDs and nineteen AEDs. Furthermore, five new serum drug panels have been introduced: antiarrhythmics, antifungals, neuroleptics, tricyclic and psychostimulant antidepressants. The immunoassays remained unchanged with respect to the previous instrumental set-up. The full list of analytes and the details of the analyzed instrumental arrangements are reported in Table 1. Data related to each test were from the laboratory database and the number of assay requests during the first semester of 2017 and 2018 was compared.

2.2. Repeatability

The coefficient of variation values (CV) of the 2017 methods for blood ISDs determination were calculated using four levels of QCs, except for CSA with only three QCs. The CV of the ISD 2018 methods were calculated with the same QCs of 2017, while three specific QCs were used for AEDs. For each QC, twenty repetitions carried out over twenty consecutive working days, were considered.

2.3. Limits of quantification

Lower limit of quantification (LLOQ) for CSA in LC-MS was verified by testing four aliquots of a blood pool, with CSA concentration corresponding to the LLOQ declared in the kit ($10 \mu g L^{-1}$), in eight replicates for four consecutive days on two different LC-MS systems. LLOQ of the LC-MS AED panel were calculated according to the CLSI EP17-A2 guidelines [18]: five different dilutions of the dedicated QC were prepared with a negative plasma pool, divided into four aliquots and analyzed in five replicates on four consecutive days. To take into account the analytical variability of laboratory routine, samples preparation was carried out by two different operators and the instrumental analysis was performed using two different LC-MS instruments. The average concentration of more diluted solution with a CV equal or lower than 20% was taken as LLOQ.

2.4. Total Value of Ownership analysis

The average TAT for blood ISDs (CSA, EVER, TACR and SIR) and for six representative AEDs was obtained considering the interval from sample check-in to result validation. For AEDs, at least one analyte was chosen for each kit used in the previous laboratory set-up: TOP, LEV, MHD, LAM, LAC and RUF. Direct observations as well as time and motion studies on the preparation and testing processes were conducted during five consecutive working days, mainly focusing on ISDs. Data include the total DLT from laboratory technicians, testing automation time and TAT for a patient sample. The DLT summarizes the total labor required for a technician throughout the sample testing process, comprising pre-analytical sample preparation steps, loading the instrument, setting up an analysis run, and post-analytical resulting steps. The DLT was calculated, for each analytical step, by subtracting all the multiples of 15 min in which the process was fully automated and there was no need for intervention by laboratory personnel. Any continuous automation cycle longer than 15 min was considered walk-away time, and was not counted. *Total Cycle Time* represents the total actual time required to complete each step of the testing and it is utilized to calculate the total TAT for a specimen or batch, and accounts for any parallel processes. The timing of different steps was monitored in both previous and new instrumental set-up for a standard batch of 51 patient samples and 4 QCs, which was derived based on the annual test volumes. The total testing process was divided into four phases: specimen acceptance, sample preparation, instrumental analysis and post-analytical phase. According to these data, TAT is measured from the start of specimen preparation to resulting, and it includes all direct labor and walk-away automation time, as well as any periods of waiting between steps.

2.5. Overall Equipment Effectiveness

To evaluate the actual utilization of the three newly installed LC-MS instruments and the ASPS, OEE indicator was applied. The observations were made for five consecutive working days and the following parameters were considered: start and end time of activity, maintenance time, samples preparation time, calibrators and QC analysis time, standby times and any machine downtime due to failures. Finally, the number of processed samples and the number of repetitions carried out for each instrument was recorded. The OEE indicator was obtained using the following formula:

$$OEE = D \times P \times Q$$

Where: i) *D (Availability)* = Net Operating Time/Operating Time; ii) *P (Performance)* = Value Time/Net Operating Time; iii) *Q (Quality)* is the number of successful samples out of the total number of samples. Further explanations about OEE calculation are available in Ref. [19].

2.6. Cost analysis

The estimation of total costs of ISD assays was made taking into account as cost items the labor data detailed above, reagents and consumables, in-service instrumentation and laboratory personnel. Consumable and reagents costs have been divided by the number of tests performed, obtaining the cost of the reagents per single test. The monthly costs of equipment (ASPS and computer, LC-MS and computer, work desk, nitrogen generator, printer) were divided by the average number of samples performed monthly, resulting in an estimation of the cost of instrumentation per test. The cost of personnel per batch was obtained considering the DLT for a standard batch and multiplying it by the hourly cost of personnel. Finally, by adding the costs of reagents, instruments and personnel, it was possible to estimate the cost for a batch of samples and, consequently, for each single test.

Table 1
Instrumental details of TDM dosages performed at the "Baldi & Riberi" laboratory with the 2017 and 2018 configurations.

	ANALYTE	ABBREVIATION	KIT	INTRUMENT
2017 CONFIGURATION	Tacrolimus	TACR	Masstrak Immunosuppressants Xe	UHPLC-MS/MS Aquity TQD (Waters)
	Everolimus	EVER	MassTox® Immunosuppressants in whole blood	HPLC-MS/MS Allians 2695 (Waters)
	Sirolimus	SIR	MassTox® Immunosuppressants in whole blood	
	Mycophenolic Acid	MPA	Mycopholic Acid Plasma/Serum	HPLC-UV Prominence (Shimadzu)
	Carbamazepine	CARB	Antiepileptic Drugs in Serum/Plasma - HPLC	
	Oxcarbamazepin	MHD	Antiepileptic Drugs in Serum/Plasma - HPLC	
	Ethosuximide	ETO	Antiepileptic Drugs in Serum/Plasma - HPLC	
	Phenytoin	FENT	Antiepileptic Drugs in Serum/Plasma - HPLC	
	Phenobarbital	FENB	Antiepileptic Drugs in Serum/Plasma - HPLC	
	Lamotrigine	LAM	Antiepileptic Drugs in Serum/Plasma - HPLC	
	Primidone	PRM	Antiepileptic Drugs in Serum/Plasma - HPLC	
	Zonisamide	ZON	Antiepileptic Drugs in Serum/Plasma - HPLC	
	Felbamate	FEL	Rufinamide, Felbamate and Lacosamide in Serum/ Plasma – HPLC	
	Lacosamide	LAC	Rufinamide, Felbamate and Lacosamide in Serum/ Plasma – HPLC	
	Rufinamide	RUF	Rufinamide, Felbamate and Lacosamide in Serum/ Plasma – HPLC	
	Levetiracetam	LEV	Levetiracetam Keppra®	
	Cyclosporin	CSA	Flex reagent cartridge CSA	Dimension Vista 1500 (Siemens)
	Valproic Acid	VALP	Flex reagent cartridge VALP	
	Carbamazepine in emergency	CARB emerg	Flex reagent cartridge CRBM	
	Phenobarbital in emergency	FENB emerg	Flex reagent cartridge PHNO	
	Phenytoin in emergency	FENT emerg	Flex reagent cartridge PTN	
	Topiramate	TOP	Topiramate Assay	
	Amikacin	AMK	Flex reagent cartridge AMK	
	Gentamicin	GNM	Flex reagent cartridge GNM	
	Teicoplanin	TCP	Flex reagent cartridge TPC	
	Vancomycin	VNC	Flex reagent cartridge VNC	
	Benzodiazepines Panel	BENZ	EMIT II Plus Benzodiazepine Assay	
2018 CONFIGURATION	Cyclosporin*	CSA*	MassTox® Immunosuppressants ONE minute	HPLC Nexera X2 - 4500MD (Shimadzu - Sciex)
	Tacrolimus*	TACR*	MassTox® Immunosuppressants ONE minute	
	Everolimus*	EVER*	MassTox® Immunosuppressants ONE minute	
	Sirolimus*	SIR*	MassTox® Immunosuppressants ONE minute	
	Mycophenolic Acid	**MPA**	MassTox® TDM Series A	
	Brivaracetam	BRIV	MassTox® TDM Series A	
	Oxcarbamazepin	**MHD**	MassTox® TDM Series A	
	Gabapentin	GAB	MassTox® TDM Series A	
	Ethosuximide	**ETO**	MassTox® TDM Series A	
	Felbamate	**FEL**	MassTox® TDM Series A	
	Lacosamide	**LAC**	MassTox® TDM Series A	
	Lamotrigine	**LAM**	MassTox® TDM Series A	
	Levetiracetam	**LEV**	MassTox® TDM Series A	
	Perampanel	PER	MassTox® TDM Series A	
	Pregabalin	PREG	MassTox® TDM Series A	
	Primidone	**PRM**	MassTox® TDM Series A	
	Retigabine	RET	MassTox® TDM Series A	
	Rufinamide	**RUF**	MassTox® TDM Series A	
	Stiripentol	STIR	MassTox® TDM Series A	
	Topiramate	**TOP**	MassTox® TDM Series A	
	Tiagabinae	TIAG	MassTox® TDM Series A	
	Vigabatrin	VIG	MassTox® TDM Series A	
	Zonisamide	**ZON**	MassTox® TDM Series A	
	Benzodiazepines Panel	**BENZ**	MassTox® TDM Series A	
	Antiarrhythmics Panel		MassTox® TDM Series A	
	Antifungals Panel		MassTox® TDM Series A	
	Neuroleptics Panel		MassTox® TDM Series A	
	Tricyclic Antidepressants Panel		MassTox® TDM Series A	
	Psychostimulant Antidepressants Panel		MassTox® TDM Series A	
	Valproic Acid	VALP	Flex reagent cartridge VALP	Dimension Vista 1500 (Siemens)
	Carbamazepine in emergency	CARB emerg	Flex reagent cartridge CRBM	
	Phenobarbital in emergency	FENB emerg	Flex reagent cartridge PHNO	
	Phenytoin in emergency	FENT emerg	Flex reagent cartridge PTN	
	Amikacin	AMK	Flex reagent cartridge AMK	
	GNM	GNM	Flex reagent cartridge GNM	
	TCP	TCP	Flex reagent cartridge TPC	
	VNC	VNC	Flex reagent cartridge VNC	

*Prepared with automatic sample preparation system.
In bold analytes for which the dosage was transferred to LC-MS.
Underlined analytes introduced with the new configuration.

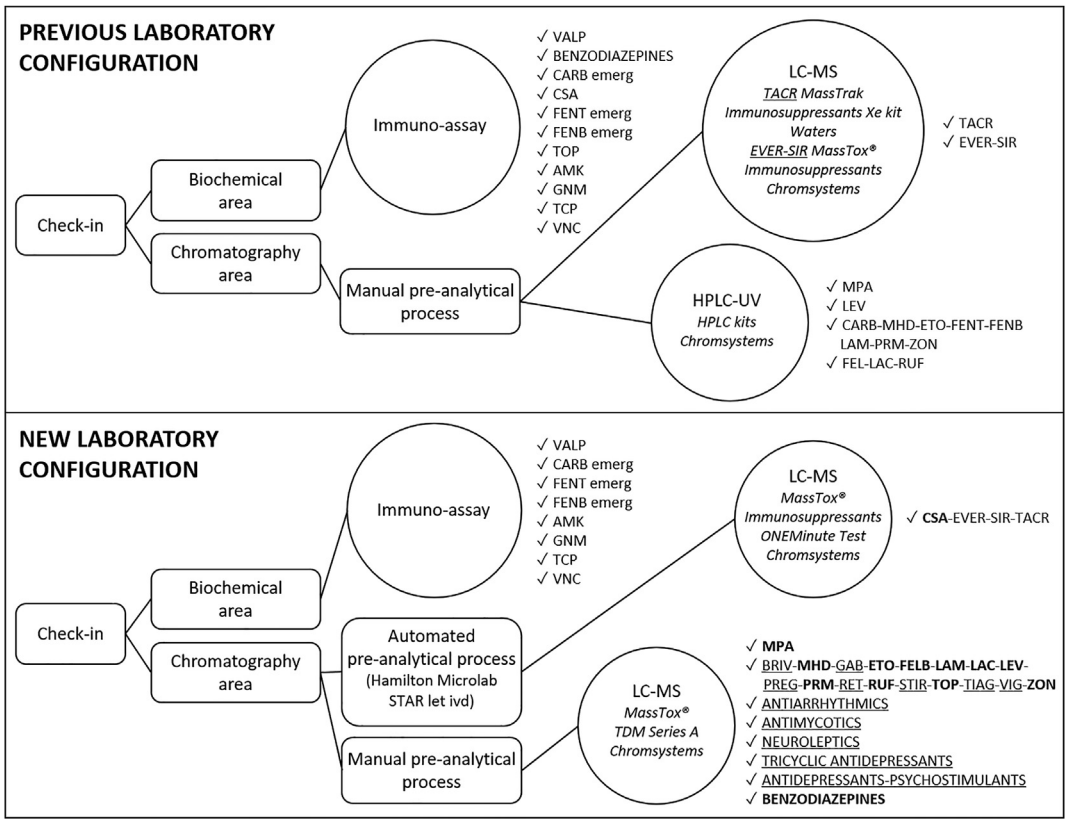

Fig. 1. Workflow organization for TDM analysis describing instrumental structure of I semester 2017 and I semester 2018. Each item in the bulleted list corresponds to a different kit/parameter set. The analytes for which, in the new instrumental set-up, the dosage has been transferred to an LC-MS method are in bold, In the analytes or classes of drugs introduced with the new configuration are underlined.

2.7. Statistical analysis

The statistical analyses were carried out using the GraphPad Prism software version 6.01 (GraphPad Software, La Jolla, CA, USA). The Kruskal-Wallis statistical test, for non-parametric data, was used to evaluate possible differences among groups of data. The Dunn test was used as a second-level test to determine which groups had a statistically significant difference with a p value < 0.05.

3. Results

3.1. Laboratory organization

The comparison between the previous and new configurations of the TDM is schematized in Fig. 1. The new configuration has provided six new AED analytes (BRIV, GAB, PREG, RET, STIR and TIAG) and five new panels of drugs: antiarrhythmics, antifungals, neuroleptics, tricyclic antidepressants and psychostimulant antidepressants. Furthermore, CSA and TOP tests were transferred to LC-MS, while serum benzodiazepines switched from a semi-quantitative method to a LC-MS quantitative method. This new layout facilitated samples' preparation: two kits used for ISDs on blood and for MPA in plasma instead of four as in the previous set-up. Regarding AEDs, five immunometric and three HPLC kits were previously required, compared to four immunometric and one LC-MS kits currently in use. In the first semester of 2017, 33,031 TDM tests were performed, 11,818 (36%) run by immunoassays and 21,213 (64%) by chromatography. In the first semester of 2018 there was an 8.7% increase (n = 35,936) in TDM workload, partly due to the introduction of new tests, with a decrease of immunoassays (−39%) and an increase of chromatographic methods (+35%) (details in Table S1 and Fig. S1 of Supplementary Material). In the first semester of 2017, the laboratory received 20,925 requests for ISDs analyses, corresponding to 22,566 tests, since 1641 requests (7.8%) included more than one ISD. Among these, 369 included both CSA (immunoassay) and at least one between EVER, TACR or SIR (LC-MS), consequently requiring different tubes to be processed in two sectors. The new approach involves a single preparation for all ISDs on blood in LC-MS and therefore only one blood tube is needed, resulting in a reduction of the collected blood amount for about 1.8% of patients. Moreover, it avoids double sample preparation for about 6% of the samples. Similarly, requests for AED included more than one drug in 692 (16%) cases and among them 147 (3.4% of the total requests) needed a double preparation for HPLC analysis, no longer necessary with the new configuration.

3.2. Repeatability

CVs obtained in the laboratory routine for ISD assays were all less than 10%, as indicated in the recommendations for the assay of ISD [7]. CVs of AED analyses also met the 15% limit set by FDA 2015 guidelines [20] (details in Tables S2 and S3 of Supplementary Material).

3.3. Limits of quantification

LLOQs declared by kits manufacturers were confirmed by laboratory verification tests for 8 analytes, while LLOQs higher than the one stated in the kit were observed for 11 analytes. The

obtained LLOQ values were below the lower limit of the reference range, with the exception of STIR and TIAG assays (details in Table S4 of Supplementary Material).

3.4. Total Value of Ownership analysis

The average TAT values as extracted by laboratory database for ISD and AED in the first semester of 2017 and 2018 are presented in Table 2. An increase of about 1 h was observed for all ISDs in the new laboratory configuration. The maximum increase was observed for CSA as a consequence of the switch from random-access immunoassay to a LC-MS method with automated sample preparation procedure. In Fig. 2 it is possible to observe that the increase in TAT for CSA is mainly due to the automated sample preparation step in the immunoassay analysis. On the contrary, in the LC-MS method this step takes up to 80 min, in which a solid phase extraction is performed employing the MassTox® Immunosuppressants ONE minute kit (Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany). Furthermore, immunoassay analysis was available 24/7 thanks to the random-access system and the automation, while it was decided to make available the LC-MS analysis daily 7/7 after agreeing with clinicians a novel workflow demanding sample collection and transfer to the laboratory before 2 p.m. each day. On the other hand, a reduction in TAT for AEDs was observed for all the analytes, except for TOP previously performed by immunoassay 24/7. Differences of TAT for AEDs monitoring by HPLC are due to the different frequency of kits used, according to different number of requests for individual analytes. The time necessary to complete each step of ISD analysis performed by LC-MS in the previous and new laboratory configurations were measured together with the relative DLT in Fig. 3 (details in Table S5, Supplementary Material). The *Total Cycle Time* for a standard batch decreased from 333 to 235 min thanks to the novel organization. It was estimated that 0.98 h represented the actual time during which the intervention of laboratory personnel is necessary (Table S6, Supplementary Material). According to TAT, it can be estimated that with the new configuration, based on LC-MS analysis, a sample batch initiated at 8:00am will end at 1:30 p.m. (Fig. 2).

3.5. Overall Equipment Effectiveness

OEE measures opportunities for improvement and is an indicator of capacity for bottleneck operations. The closer the value is to 1, the more it means that the instrument/analyzer provides only correct results, without repetition, in the shortest possible time and without breaks or loss of time. OEE analysis was performed on the ASPS and the two LC-MS systems dedicated to blood ISDs (LCMS-ISD) and to other TDM analyses (LCMS-TDM). As shown in Fig. 4 and Table 3, the ASPS instruments and the two LCMS-ISD systems demonstrated higher D values ($\mu = 0.92$ and $\mu = 0.86$, respectively) with respect to LCMS-TDM ($p < 0.0001$). On the contrary, the highest P value ($\mu = 0.78$) was obtained for LCMS-TDM instrument, without significant difference with LC-MS-ISD ($\mu = 0.51$) and ASPS ($\mu = 0.67$). All systems had good Q values, meaning that only good results are being produced, including samples that need re-run. The results obtained indicate that there is a margin of recovery in terms of operations both in the preparatory phase (HAM), which may represent a bottleneck, and in the analytical phase for instruments dedicated to the analysis of ISD.

Table 2
Comparison of Total Analysis Time (TAT) of ISD blood and AED analyses in first semester of 2017 and 2018.

Assay	TAT Jan–May 2017	TAT Jan–May 2018
CSA	2.3 h	3.6 h
EVER-TAC-SIR	2.7 h	3.6 h
ISD blood total	2.6 h	3.6 h
TOP	4.8 h	1.4 days
LEV	2.5 days	1.4 days
MHD-LAM	3.1 days	1.4 days
LAC-RUF	4.3 days	1.4 days
AED total[a]	2.5 days	1.4 days

[a] TOP, LEV, MHD-LAM, LAC-RUF

3.6. Cost analysis

The cost analysis, performed for ISD tests (Table 4), compared the unit costs and the costs for running a standard batch with the two laboratory configurations. In the new set-up, the total cost of in-service instrumentation amounts to 8751 euro/month, which gives a cost of 2.63 euro/sample, resulting in a costs reduction of 51.2% compared to the former configuration. From the sum of all costs indicated, the cost of a batch is 336.84 euro (6.60 euro per reportable result), which means a costs reduction of 56.1%

4. Discussion

The comparison between the two configurations of TDM work area showed a reduction in number of kits used, made possible by availability on the market of new kits for wider multi-parametric analysis and sample preparations for both ISDs and AEDs. Moving CSA test from immunoassay to LC-MS and measuring of all four ISDs in a single run brought significant advantages in selectivity and increased adherence between therapy and TDM required [7]. Similarly, the shift from three HPLC to a single LC-MS kit, requiring fewer purification steps, allowed to reduce both number and duration of preparations. For instance, HPLC kit for LEV dosage included an extraction procedure particularly time-consuming because of several columns' conditioning and washing steps, while the currently used LC-MS kit consists of a protein precipitation followed by dilution of the supernatant. On the other hand, despite the availability of one kit for simultaneous determination of CARB, FENB, FENT and VALP, these tests were maintained on the immunomediated platforms considered adequate during previous years, thus assuring urgency and result delivery 24/7 [21]. Furthermore, it was only possible to analyze VALP with single mass, because of its chemical-physical characteristics, resulting in minor advantages in selectivity. Since the CV values calculated for new AED methods were in line with those declared by kit manufacturers and with the repeatability requirements of 2015 FDA guidelines [20], the implemented methods were judged fit for purpose. Although new ISD methods met repeatability requirements of the ISD dosages guidelines [7], CV values obtained for CSA, EVER and SIR were slightly higher than those provided by manufacturer. This discrepancy can be explained by different periods of such evaluation (10 vs 20 days by manufacturers' and our experiments, respectively). In addition, our analysis was performed under routine conditions using three LC-MS instruments, while the number of platforms used for kits' validation was not specified by manufacturers. LLOQs verification showed the need of further analysis for STIR and TIAG, for which the calculated LLOQs were higher than the lower value of the reference range. LLOQs values higher than those provided by the manufacturer but lower than the reference range limit, were considered acceptable because such differences could be accounted by pre-analytical variability associated with different factors, such as operators and platforms. One main advantage of the new laboratory configuration for AEDs monitoring is represented by the reduction of TAT for all AEDs tests except TOP. This decrease is particularly important for low-demand

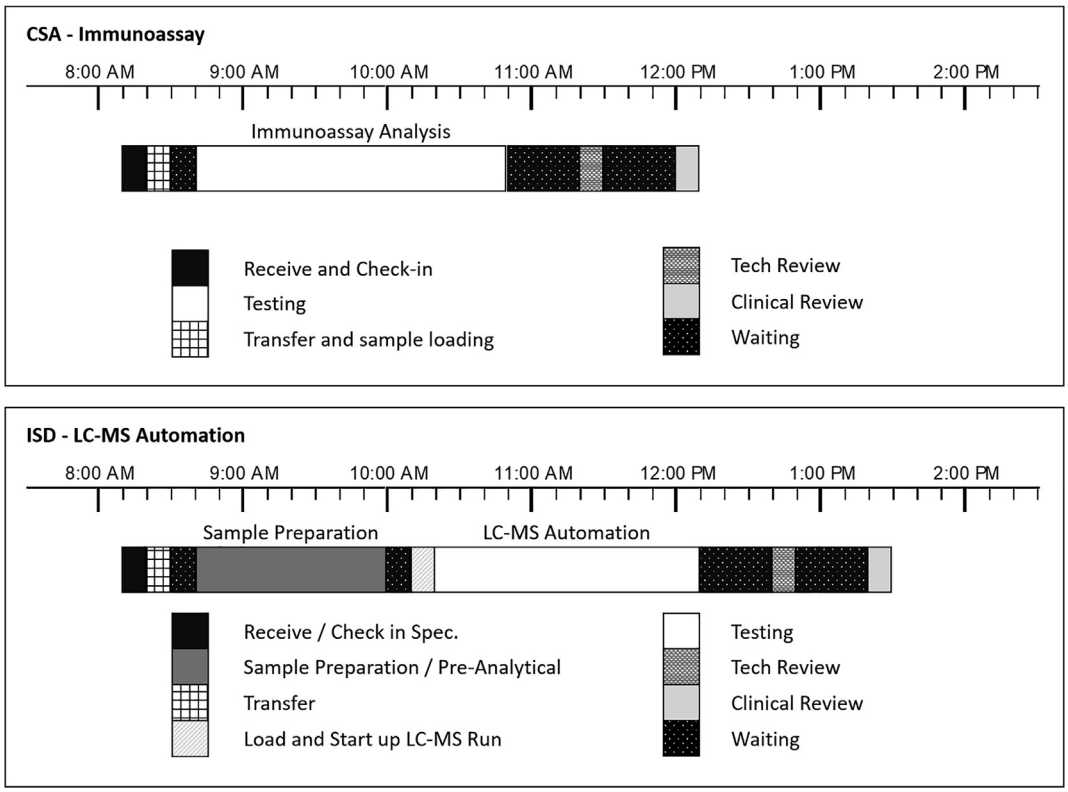

Fig. 2. Details of Turnaround Time (TAT) for an example batch of CSA analysis with the immunoassay and the LC-MS method.

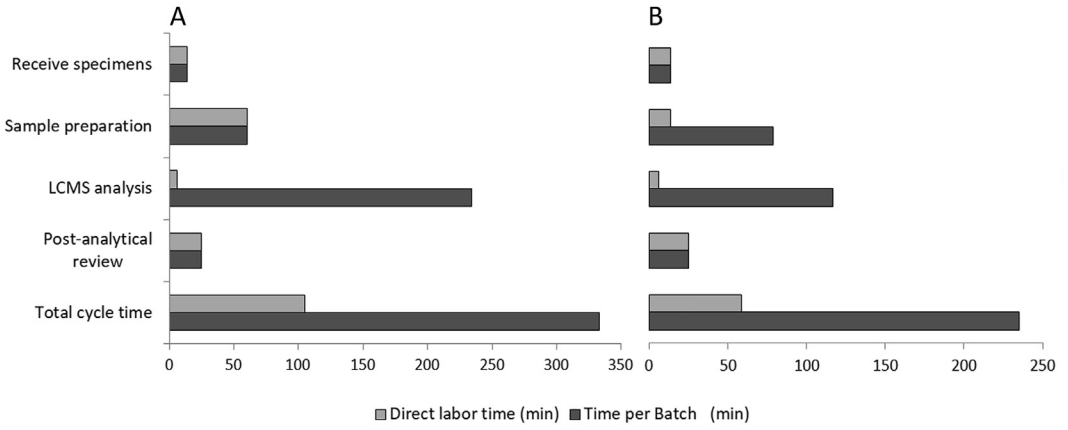

Fig. 3. Total Cycle Time and Direct Labor Time (DLT) for a typical batch consisting of 51 patient samples and 4 quality controls measured with the laboratory configuration of 2017 (A) and 2018 (B).

drugs, such as LAC, RUF and FEL (from 4.3 to 1.4 days on average). Otherwise, the average TAT for ISD increased from 2.6 h to 3.6 h, confirming that the use of tools that reduce manual processes do not necessarily reduce process times [22]. Indeed, *Total Cycle Time* and DLT assessment showed that, for a standard batch, the operator is engaged only for 25% of the total analysis time. In our experience the calculation of high-level time summary for the preparation and testing for ISD specimens using the LC-MS systems gave us useful tools to standardize processes as well as to improve the laboratory workflow. The thorough analysis performed represents an example to show how to use the numbers to calculate time or cost for each test.

The OEE analysis found high D values for ASPS and the two LCMS-ISDs, while the highest P value was obtained for LCMS-TDM system. A possible explanation for these differences can be related to the fact that ASPS and LCMS-ISD instruments are dedicated to a single class of analytes (ISD), hence reducing the set-up times, whereas LCMS-TDM, processing different types of samples (e.g., MPA, AED and benzodiazepines), needs more set-up time. ASPS and LCMS-ISD are more often running during planned production time, with less stops. The P parameter, which decreases with the increase of stand-by times, was found to be higher for the LCMS-TDM instrument, even if it processes less samples on average. This can be explained in part by the different duration of analytical runs (5 vs 1.5 min for AED and ISD analysis, respectively). In fact, being constant the number of analyzed samples, LCMS-TDM instrument had less instrumental stand-by time and therefore is running as fast as possible. In addition, ASPS and LCMS-ISD, working according to

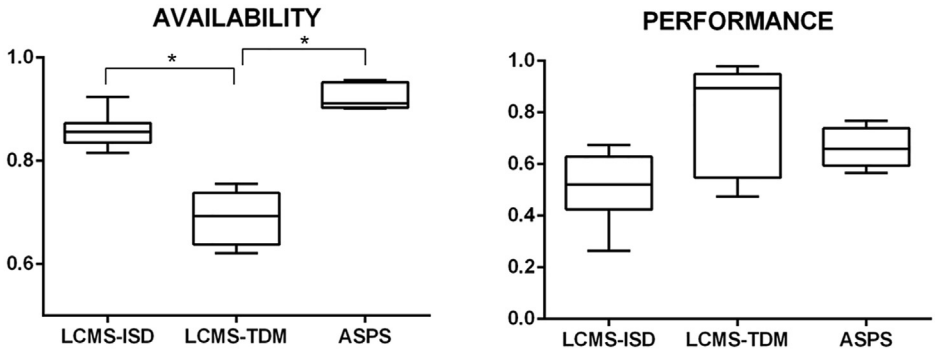

Fig. 4. Box-plot of Availability (D) and Performance (P) values for LCMS-ISD, LCMS-TDM and ASPS. Statistically significant differences are indicated with an asterisk.

Table 3
Mean values* of the terms of OEE analysis for LCMS-ISD, LCMS-TDM and ASPS assays.

Assay	D [μ]	P [μ]	Q [μ]	OEE [μ]	N° day
LCMS-ISD (n=2)	0.86 (0.82–0.92)	0.51 (0.26–0.67)	0.97 (0.86–1.00)	0.42 (0.21–0.57	86.2 (42–127)
LCMS-TDM	0.69 (0.62–0.76)	0.78 (0.47–0.98)	1.00 (1.00–1.00)	0.54 (0.36–0.68)	48.6 (28–76)
ASPS	0.92 (0.90–0.96)	0.67 (0.57–0.77)	0.99 (0.99–1.00)	0.61 (0.50–0.69)	175 (148–211)

* minimum and maximum values in brackets.

Table 4
Comparison of costs for ISD testing with the 2017 and 2018 laboratory configurations.

Cost item	Previous laboratory configuration			New laboratory configuration		
	Unit cost	Quantity	Total per batch*	Unit cost	Quantity	Total per batch*
Equipment per sample**	€ 6.14	51	€ 313.14	€ 2.63	51	€ 133.89
Consumables per test	€ 7.39	55	€ 406.45	€ 3.27	55	€ 179.78
Consumables per batch	–	–	–	€ 2.08	1	€ 2.08
Quality controls	€ 4.25	4	€ 17	€ 1.15	4	€ 4.60
Direct Labor Time [h]	€ 16.83	1.75	€ 29.45	€ 16.83	0.98	€ 16.49
Total per batch			€ **766.04**			€ **336.84**
Average per result			€ **15.02**			€ **6.60**

*The calculation is based on a batch consisting of 51 patient samples and 4 QCs.
**Equipment monthly cost based on 3333 patient samples per month (samples per month year 2017).

sample arrival flow, are subject to downtime, while LCMS-TDM, working mainly on samples arrived the day before or during the week, allows a better organization and the reduction of stand-by time. The obtained results indicated that there is a recovery margin in terms of operations both in the preparatory phase (ASPS), which may represent a bottleneck, and in the analytical phase for LCMS-ISD. Optimization of the sample arrival and reception could improve instrument utilization, by reducing stand-by time. The automated preparation step should be extended either running more sessions in a day or duplicating the system. An additional ASPS would also allow expanding the automated preparation to other analytes, bringing further advantages in terms of sample traceability and data repeatability.

5. Conclusions

The present work assessed with an integrated approach potentiality and critical issues related to the implementation of a novel laboratory organizational model aimed at the LC-MS automation. TVO is a methodology for measuring and analyzing business value of investments that considers the benefits of alternative choices. We performed this analysis in a clinical laboratory setting through a comparative measurement that evaluates benefits in terms of flexibility and standardization of a new model of LC-MS area. The employed TVO model may take a holistic view of the new solution and could be used in the future as product/process comparison tool in other laboratories. Indeed, this cost-benefit analysis framework quantifies the benefits of laboratory solutions, such as enhanced productivity and lower clinical risks.

The OEE analysis, employed in the industrial context, proved to be applicable to analytical processes, even in specialized areas that need the control of specialized operators. This approach highlighted the steps causing productivity reduction of the whole laboratory configuration, also indicating possible process improvements. In particular, we focused on making sample processing flow more efficient, from the pre-analytical phase to the management of workload on multiple platforms. The main limitations of the study derive from the low number of observation days for OEE analysis that, therefore, could not represent the entire case study of any extraordinary or unforeseen maintenance of a different nature. In perspective, the future growing demand for rapid response from specialist sectors, such as TDM, will be associated with an increase of training and initial costs. Therefore, to continue creating value while not nicking economic sustainability, laboratories will have to optimize their processes introducing increasingly automation levels. In the field of MS-based assays, two different types of platforms will be present in the near future: one similar to the current system, very flexible and aimed at research and relatively low volumes tests, and a second one with a high level of automation designed for high volume routine tests, such as

vitamin D and ISDs analysis. As demonstrated by this study, automation developments will also impact on costs, allowing the reinvestment of available resources in research activities for technical improvement.

CRediT authorship contribution statement

Fabio Settanni: Conceptualization, Supervision, Writing – review & editing. **Federico Ponzetto:** Writing – original draft, Writing – review & editing. **Agnese Veronesi:** Validation. **Antonello Nonnato:** Supervision, Data curation. **Francesco Martinelli:** Validation. **Francesca Rumbolo:** Data curation, Writing – review & editing. **Maurizio Fimognari:** Validation. **Giovanna Martinasso:** Supervision, Writing – review & editing. **Giulio Mengozzi:** Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2021.338455.

References

[1] C.E. Pippenger, Therapeutic drug monitoring assay development to improve efficacy and safety, Epilepsy Res. 68 (2006) 60–63.
[2] M. Eichelbaum, M. Ingelman-Sundberg, W.E. Evans, Pharmacogenomics and individualized drug therapy, Annu. Rev. Med. 57 (2006) 119–137.
[3] D.L. Wyles, J.G. Gerber, Antiretroviral drug pharmacokinetics in hepatitis with hepatic dysfunction, Clin. Infect. Dis. 40 (2005) 174–181.
[4] M. Mirochnick, E. Capparelli, Pharmacokinetics of antiretrovirals in pregnant women, Clin. Pharmacokinet. 43 (2004) 1071–1087.
[5] A. Morandi, G. Bellelli, E.E. Vasilevskis, R. Turco, F. Guerini, T. Torpilliesi, et al., Predictors of rehospitalization among elderly patients admitted to a rehabilitation hospital: the role of polypharmacy, functional status, and length of stay, J. Am. Med. Dir. Assoc. 14 (2013) 761–767.
[6] G. Brandhorst, M. Oellerich, G. Maine, P. Taylor, G. Veen, P. Wallemacq, Liquid chromatography-tandem mass spectrometry or automated immunoassays: what are the future trends in therapeutic drug monitoring? Clin. Chem. 58 (2012) 821–825.
[7] C. Seger, M. Shipkova, U. Christians, E.M. Billaud, P. Wang, D.W. Holt, et al., Assuring the proper analytical performance of measurement procedures for immunosuppressive drug concentrations in clinical practice: recommendations of the international association of therapeutic drug monitoring and clinical toxicology immunosuppressive drug scientific committee, Ther. Drug Monit. 38 (2016) 170–189.
[8] I. Sketris, R. Yatscoff, P. Keown, D.M. Canafax, M.R. First, D.W. Holt, et al., Optimizing the use of cyclosporine in renal transplantation, Clin. Biochem. 28 (1995) 195–211.
[9] A. Hamwi, A. Salomon, R. Steinbrugger, M. Fritzer-Szekeres, W. Jäger, T. Szekeres, Cyclosporine metabolism in patients after kidney, bone marrow, heart-lung, and liver transplantation in the early and late posttransplant periods, Am. J. Clin. Pathol. 114 (2000) 536–543.
[10] N.W. Brown, C.E. Gonde, J.E. Adams, J.M. Tredger, Low hematocrit and serum albumin concentrations underlie the overestimation of tacrolimus concentrations by microparticle enzyme immunoassay versus liquid chromatography-tandem mass spectrometry, Clin. Chem. 51 (2005) 586–592.
[11] M. Vogeser, C. Seger, A decade of HPLC-MS/MS in the routine clinical laboratory-goals for further developments, Clin. Biochem. 41 (2008) 649–662.
[12] M. Marinova, C. Artusi, L. Brugnolo, G. Antonelli, M. Zaninotto, M. Plebani, Immunosuppressant therapeutic drug monitoring by LC-MS/MS: workflow optimization through automated processing of whole blood samples, Clin. Biochem. 46 (2013) 1723–1727.
[13] L.M. Ellram, A framework for total cost of ownership, Int. J. Logist. Manag. 4 (2) (1993) 49–60.
[14] V. Buljanović, H. Patajac, M. Petrovecki, Clinical laboratory as an economic model for business performance analysis, Croat. Med. J. 52 (2011) 513–519.
[15] P. Valenstein, Laboratory turnaround time, Am. J. Clin. Pathol. 105 (1996) 676–688.
[16] Y. Gendre, G. Waridel, M. Guyon, J.F. Demuth, H. Guelpa, T. Humbert, Manufacturing execution systems: examples of performance indicator and operational robustness tools, Chimia 70 (2016) 616–620.
[17] S. Nakajima, Introduction to TPM: Total Productive Maintenance (Preventative Maintenance Series), Productivity Press, Cambridge, MA, 1988.
[18] Evaluation of detection capability for clinical laboratory measurement procedures; approved guidelines – second edition, Clinical and Laboratory Standard Institute. Available at: https://clsi.org/media/1430/ep17a2_sample.pdf.
[19] P. Muchiri, L. Pintelon, Performance measurement using overall equipment effectiveness (OEE): literature review and practical application discussion, Int. J. Prod. Res. 46 (2008) 3517–3535.
[20] Food Drug Administration, Bioanalytical method validation guidance, Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf.
[21] A.H. Wu, C. McKay, L.A. Broussard, R.S. Hoffman, T.C. Kwong, T.P. Moyer, et al., National Academy of Clinical Biochemistry Laboratory Medicine. National academy of clinical biochemistry laboratory medicine practice guidelines: recommendations for the use of laboratory tests to support poisoned patients who present to the emergency department, Clin. Chem. 49 (2003) 357–379.
[22] Y.V. Zhang, A. Rockwood, Impact of automation on mass spectrometry, Clin. Chim. Acta 450 (2015) 298–303.