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Review | Обзор

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Validating Bioanalytical Methods for Biomarker Quantitation: A Regulatory Document Review

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ABSTRACT

INTRODUCTION. Biomarkers are used to assess normal physiological processes in the body; to diagnose and select therapy for various diseases; and to develop new drugs. The increasing use of biomarkers as drug development tools necessitates improvements in analytical quantification methods.

AIM. This study aimed to review and summarise data on validation of bioanalytical methods for biomarker quantification.

DISCUSSION. The analysed regulatory documents were issued by International Council for Harmonisation (ICH), as well as Food and Drug Administration (FDA), including the Biomarker Assay Collaborative Evidential Considerations Writing Group and Critical Path Institute (C-Path); European Medical Agency (EMA); Eurasian Economic Commission (EEC); and other publicly available research platforms (including PubMed, Web of Science, RSCI (e-Library), and Google Scholar online databases) primarily published in 2014–2024. Analytical and clinical biomarker validation was examined, alongside with analytical validation stages and key validation parameters of the bioanalytical method depending on the objective (analysis for pharmacokinetics, bioequivalence, and toxicokinetics studies; biomarker analysis in drug development and for diagnosis in preclinical and clinical studies). Proposed is an algorithm for choosing biomarker analytical validation level depending on the method (chromatography, ligand-binding methods using reagent kits for various purposes) and the issues to be addressed.

CONCLUSIONS. Confirming biomarker method suitability as per the objectives is similar to a common validation procedure of bioanalytical methods. A broad and detailed scientific discussion of biomarker analysis validation is needed, since a comprehensive scheme developed for this complex procedure will contribute to more reliable use of biomarkers, improved quality of studies within this process, and resulting safe and effective new medicinal products.

Keywords: biomarkers; bioanalytical method; biomaterial; analytical validation; clinical validation; validation parameters; scope of validation tests; pre-clinical study; clinical study

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Вопросы валидации биоаналитических методик количественного определения биомаркеров: обзор нормативных документов

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РЕЗЮМЕ

ВВЕДЕНИЕ. Биомаркеры используют при оценке нормальных физиологических процессов в организме; для диагностики и подбора терапии различных заболеваний; при разработке новых лекарственных препаратов. Расширение использования биомаркеров в качестве инструментов разработки лекарственных препаратов обусловливает необходимость совершенствования методов их количественного определения.

ЦЕЛЬ. Обобщение материалов по вопросам валидации биоаналитических методик количественного определения биомаркеров.

ОБСУЖДЕНИЕ. Использованы регуляторные документы Международного совета по гармонизации (International Council for Harmonisation, ICH), Управления по контролю за качеством продуктов питания и лекарственных средств (Food and Drug Administration, FDA), включая Рабочую группу по совместному обсуждению анализа биомаркеров (Biomarker Assay Collaborative Evidentiary Considerations Writing Group) и Институт критического пути (Critical Path Institute, C-Path) Европейского агентства по лекарственным средствам (European Medicines Agency, EMA), Евразийской экономической комиссии (ЕЭК) и научные публикации, находящиеся в открытом доступе (в том числе по данным библиографических баз и поисковых систем PubMed, Web of Science, РИНЦ (eLIBRARY.RU), Google Scholar), преимущественно опубликованные в период 2014–2024 гг. Рассмотрены процессы аналитической и клинической валидации биомаркеров, отмечены этапы их аналитической валидации, выделены ключевые показатели валидации биоаналитической методики в зависимости от задачи (анализ для исследований фармакокинетики, биоэквивалентности и токсикокинетики; анализ биомаркеров при разработке лекарственных препаратов и для диагностики в ходе доклинических и клинических исследований). Предложен алгоритм выбора уровня аналитической валидации биомаркеров в зависимости от особенностей метода (хроматография, лигандсвязывающие методы с использованием наборов реагентов различного назначения) и решаемых задач.

ВЫВОДЫ. Подтверждение пригодности методики анализа биомаркеров для применения согласно планируемым целям близко к общепринятой процедуре валидации биоаналитических методик. Необходимо широкое научное обсуждение деталей валидации анализа биомаркеров, поскольку выработка единого алгоритма этой сложной процедуры будет способствовать более надежному использованию биомаркеров, повышению качества сопутствующих этому процессу исследований и конечному результату — введению в обращение новых эффективных и безопасных лекарственных препаратов.

Ключевые слова: биомаркеры; биоаналитическая методика; биоматериал; аналитическая валидация; клиническая валидация; валидационные параметры; объем валидационных испытаний; доклинические исследования; клинические исследования

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Финансирование. Работа выполнена без спонсорской поддержки.

Потенциальный конфликт интересов. М.Н. Макарова — член редакционной коллегии журнала «Регуляторные исследования и экспертиза лекарственных средств» с 2018 г. Остальные авторы заявляют об отсутствии конфликта интересов.

INTRODUCTION

Biomarkers Definitions Working Group of the Food and Drug Administration (FDA) and National Institutes of Health (NIH, USA) understands biological marker as "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions"[1] [1]. In the interpretation of [2, 3] there is a slightly different emphasis on "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, disease processes, or pharmacologic responses to a therapy". The authors of [4], with reference to [3], give the following definition of a biomarker: an objectively investigated parameter, measured with high accuracy, reproducibility and reliability, which allows to reflect the intensity of physiological processes, the state of health, the risk degree or the fact of the disease, its stage, and outcome prognosis.

The parameter can be chemical, physical, or biological. Biomarkers are used in assessing body physiological processes; detecting the disease and selecting treatment; predicting the disease course, progression and outcome; developing new medicinal products, establishing their therapeutic and side effects, and identifying the mechanism of action [4]. Given the large number of various biomarkers, a single classification is not applicable. Currently, biomarkers are classified depending on their purpose in diagnosis and treatment, on the study system, type, specificity, etc.; thus, several ways to classify them exist [4]. According to one of classifications [1]:

Type 0 – a marker used to detect presence of a disease and correlating with its clinical signs.
Type I – a marker associated with drug therapeutic effect and mechanism of action.
Type II – (predictor of clinical outcome, surrogate endpoint) a marker that allows to assume the disease outcome and evaluate the treatment effectiveness.

Based on the task, the following biomarker groups are known [2]:

- diagnostic ones allow to establish the presence or absence of a condition and its severity (for example, the amount of glycated haemoglobin and diabetes mellitus);
- prognostic ones allow to assess the risk of a condition or its complications (for example, gene polymorphism of coagulation factor);
- therapeutic ones help to evaluate or predict the outcome of exposure to a drug therapy (for example, the international normalised ratio (INR) is an assessment of warfarin effectiveness, which is a standardised indicator introduced by the World Health Organisation to unify the results of a prothrombin test in different laboratories [5]).

Accepting a parameter as a biomarker is a long multi-level process requiring to analyse the results of various clinical, phylogenetic and other studies [6]. In the review [6], the authors discussed the opportunity of using several parameters (related to genes encoding various enzymes of the cytochrome P450 complex, the uridine diphosphate glucuronosyltransferase family and glycoprotein P) as pharmacogenetic markers of antiepileptic pharmacokinetics to improve the effectiveness and safety of anticonvulsant therapy. However, it was concluded that due to the controversial results and the lack of appropriate randomised prospective placebo-controlled trials, reliable data on this issue do not exist as yet.

Notably, not every laboratory parameter can be called a biomarker. According to the authors [2], the main feature of a biomarker is its statistically valid relationship with the disease, complication,

[1] US FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS and other Tools) Resource.

and therapy effect. The authors noted that only 20% of the studies they analysed[2] showed sensitivity, specificity, and prognostic value of this parameter. In most studies, the indicator is called a biomarker since it significantly increases/decreases/occurs in a certain pathology; this is clearly insufficient to recognise the parameter as a biomarker.

Given the increasing role of biomarkers in the diagnostics, therapy monitoring, and development of modern biological and pharmacological preparations, it is important to review and systematise current regulatory requirements, international, national, and scientific recommendations in order to identify and apply them.

The aim of the study is to review and summarise validation data of bioanalytical methods for biomarker assay.

MATERIALS AND METHODS

Regulatory documents of the International Council for Harmonisation (ICH), Food and Drug Administration (FDA, including Biomarker Assay Collaborative Evidentiary Considerations Writing Group and Critical Path Institute, C-Path), European Medicines Agency (EMA), Eurasian Economic Commission (EEC), and other open scientific sources (including electronic databases PubMed, Web of Science, RSCI (eLibrary), Google Scholar) mostly published over 2014–2024 were used for this study. Keywords used in queries: биомаркеры (biomarkers), аналитические/биоаналитические методы (analytical/bioanalytical methods), количественное определение (assay, quantification), валидация (validation).

RESULTS AND DISCUSSION

Clinical and analytical validation (qualification) of a biomarker. It was noted in [7] that a biomarker should be selected, studied and validated for its successful application. In this case, validation implies confirming that the selected biomarker fulfils its functions (clinical, diagnostic, etc.), and its use provides the expected results (defining the disease, assessing therapy effect on the disease course, identifying drug targets, etc.). The *Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices*[3] examines analytical validation (including all factors that belong to the analysis procedure, depend on acceptability of the samples, crucial reagents, and performance of equipment or test system, including sampling, processing, and storage) and clinical validation of a biomarker (to interpret biomarker measurements, assess clinical sensitivity, specificity, and accuracy of the biomarker in predicting the assumed result). Biomarker qualification is another common term in the literature [8], presumably synonymous with the validation; in our opinion, these both terms are attributable to clinical biomarker validation (or clinical qualification). Notably, many papers devoted to the biomarker validation discuss the aspects of clinical validation – prognostic value, biomarker response for various experimental conditions (populations, diseases, etc.) both within clinical and preclinical studies (pre-CS), as well as legal issues of protecting biological information, using biomedical techniques and biomaterials [9–16]. According to the authors [14], due to the lack of large-scale cohort studies that would confirm clinical significance of potential biomarkers, less than a hundred biomarkers out of several thousand discovered over the past decades have found their clinical use in medicine, and a little more than ten biomarkers – in the veterinary

[2] E-Library publications for 2014-2024, the search was carried out using the keywords: biomarkers, analytical/bioanalytical methods, assay, quantification, validation.

[3] Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.

medicine. Therefore, clinical validation of biomarkers is certainly noteworthy. Notably, the more generally accepted and clinically widespread an indicator, the more data (using established measurement techniques) are available and assayed in a significant number of patients and populations, the more likely it is to identify the clinical significance and qualification of the indicator as a biomarker.

At the same time, the data underlying clinical validation / qualification of a biomarker should use reliable methods (bioanalytical techniques) to measure a parameter that is a potential biomarker. Since the following descriptions are used for a biomarker: defined, objectively measured, measurement with high accuracy, reproducibility and reliability, it is obviously necessary to develop a methodology for quantifying the selected biomarker and its subsequent validation.

Regulatory aspects of bioanalytical techniques validation and biomarker analysis methods. Validation procedure and scope for bioanalytical techniques is regulated by several documents[4]. The guidelines aim to describe the validation of bioanalytical techniques used to detect concentrations of chemical and biological products and their metabolites in biological samples (blood, plasma, serum, other bodily fluids or tissues) obtained in toxicokinetic (TK) and pharmacokinetic (PK) studies and at all stages of clinical studies (CS), including comparative bioavailability/bioequivalence studies. All documents show procedural differences for chromatographic and ligand binding methods.

The Guidelines[5] apply, inter alia, to biomarker analysis techniques. Other documents[6] state that biomarker analysis and bioanalytical methods used to assess immunogenicity are not within the scope of the guidelines. The discrepancy concerning biomarkers is presumably because a full cycle of validation tests is rather resource-intensive and time-consuming, so its rationale in the early study of a potential biomarker is debatable. According to E.S. Don et al. [7], biomarker validation should first of all include quantification (a bioanalytical technique), or even develop such a technique. The biomarker study has an unknown range of variable possible concentrations, the influence of sampling conditions and storage, matrix components, and reagents used. In addition, the missing well-established reference samples for assay complicate the validation of such methods, causing lack of unified strategy or possibility to validate the method only in certain tasks, thus allowing unsuitability for alternative, other purposes [7].

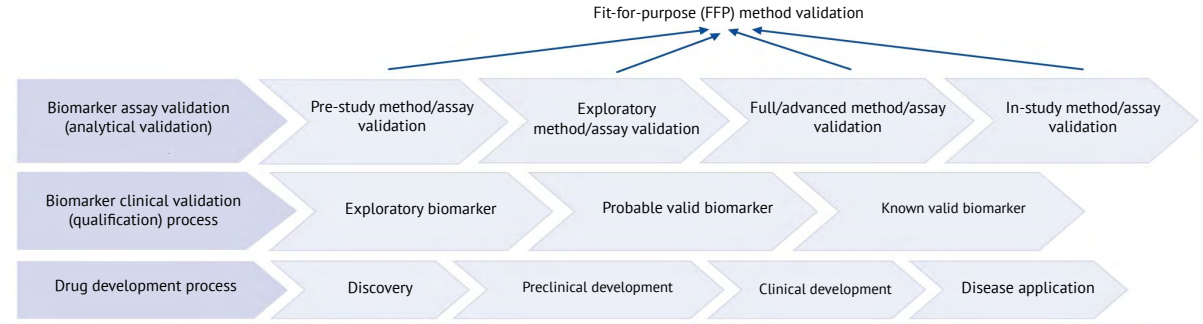
Regarding analytical techniques for determination of biomarkers in biological matrices, English-language literature establishes the context of use (COU) and fit-for–purpose (FFP)[7], implying that preliminary results of a potential (prospective) biomarker assessment can be used for exploratory research, while more essential decisions are based on data on a validated (qualified) biomarker [17]. Therefore,

[4] Decision of EEC Council dated 03.11.2016 No. 85 Rules for conducting bioequivalence studies of medicines within Eurasian Economic Union.
Guidance for industry: Bioanalytical method for validation. Rockville, 2018.
Guideline on bioanalytical method validation. EMEA/CHMP/EWP192217/2009. London, 2011.
ICH M10 on bioanalytical method validation. Scientific guideline. EMA/CHMP/ICH/172948/2019. Amsterdam, 2022.

[5] Guidance for industry: Bioanalytical method for validation. Rockville, 2018.

[6] Guideline on bioanalytical method validation. EMEA/CHMP/EWP192217/2009. London, 2011.
ICH M10 on bioanalytical method validation. Scientific guideline. EMA/CHMP/ICH/172948/2019. Amsterdam, 2022.

[7] Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.



The figure is prepared by the authors

Fig. 1. *Integrating analytical validation of biomarker analysis and its clinical qualification with drug development (based on moderated material [18])*

Table 1. *Key elements of bioanalytical method validation depending on the objective*

Parameters	Bioequivalence, pharmaco- and toxicokinetics	Biomarker analysis during drug development		Biomarker analysis for diagnosis
Stage of drug development, type of decision	Regulatory pre-CS and CS of drug substances	Development, screening,defining further use of the biomarker	Translational pre-CS and CS, candidate biomarker selection, safety, mechanism of action (PD), dose selection, dosing regimen, biomarker proof of concept	Pre-CS and CS, identification of diseases and pathological conditions, safety assessment of the biomarker validation for the subject/patient, confirmation of diagnosis
Validation level*	Full validation	Discovery/ exploratory validation	Translational/ partial validation	Full/advanced validation
Analyte	Exogenous, endogenous	Endogenous		
Matrix	Authentic or surrogate, parallelism/linearity of response	Authentic or surrogate, parallelism (if samples are available)	Authentic or surrogate, discussion of the disease status, several donors (at least 6 sources for chromatographic methods, at least 10 – for ligand binding methods), parallelism	
Standards	Generally well characterised	Reagents and standards should be well characterised, batch-to-batch change control established, GMP requirements met, stability of reagents ensured		
Calibration samples, QC samples	Model mixtures with analyte additives	Model mixtures with analyte additives, QC samples, can be used as real samples from animals/humans and/or lyophilised samples of 2–3 concentration levels from reagent kit		

Table 1 (continued)

Parameters	Bioequivalence, pharmaco- and toxicokinetics	Biomarker analysis during drug development		Biomarker analysis for diagnosis
Selectivity/ specificity	Analyte specificity, selectivity in the presence of unrelated impurities in the biological sample minimum 6 blank sources for chromatographic methods, at least 10 – for ligand binding methods	Analyte specificity; reference standard from reagent kit can be used		Selectivity in the presence of other isoforms, co-mediators and endogenous ligands Check the reference standard from the reagent kit
Calibration model	More often linear (chromatography), mathematic model selection (ligand binding methods)	Mathematic model selection (ligand binding methods), less often linear (chromatography)		
Calibration (analytical) range	Not less than 6 concentration levels, 3 replicates for chromatographic methods, 6 – for ligand binding methods	Not less than 6 concentration levels, 3 replicates		Not less than 6 concentration levels, 6 replicates
Sensitivity	LLOQ is determined using acceptance criteria (minimum calibration standard, 3 replicates for chromatographic methods, 6 – for ligand binding methods); LOD can be determined as well (more often used for ligand binding methods, the scheme from the instructions for the commercial reagent kit and/or calculated by the ratio of the average value of the analytical signal and standard deviation, SD) For chromatography, the analytical signal for the LLOQ level is at least 5 times greater than the signal of the blank sample			
Validation of accuracy** and precision***	4 QC levels, 3 replicates, not less than 2 different days for chromatographic methods, 5 QC levels, 6 replicates, not less than 2 different days for ligand binding methods)	No recommendations or without specifying the number of levels, 3 replicates	According to various sources: 4–5 QC levels, 2 replicates, 2–6 levels, 3–6 replicates or 4–6 levels, 6 replicates	By various sources: 4 QC levels, 3 replicates for chromatographic methods, 5 QC levels, 6 replicates for ligand binding methods, 4–6 levels, 6 replicates
Stability****	Stock and working solution of analyte and IS freezing/thawing, short-term and long-term stability, matrix spiked samples	Short-term stability, can include room temperature or storage in the refrigerator (up to 4 h), 1 defrosting/ freezing cycle	According to various sources: stability of reagents, freezing/thawing (3 cycles), short-term (room temperature, refrigerator – up to 24 hours) and long-term stability (–20 °C and/or –80 °C, 1–2 years, samples with additives to the matrix and/or analysed samples	

Parameters	Bioequivalence, pharmaco- and toxicokinetics	Biomarker analysis during drug development		Biomarker analysis for diagnosis
Parallelism, minimum required dilution, dilution linearity	Required mainly for ligand binding methods (linearity of dilution is also relevant for chromatographic methods, at least 5 determinations per dilution, accuracy and precision within ±15% recommended)			
Acceptance criteria	Acceptance criteria are established by regulatory documents	Acceptance criteria are not required, based on the assessment results	Acceptance criteria are based on the results of the assessment and technologically sound analytical considerations (including CI, 2SD, etc.), can be approximated and/or similar to those adopted for BE	
Documents	Validation plan/report	Description of analysis and results, instructions for commercial reagent kits	Validation plan/report	
Regulatory requirements	Compliance with GLP principles	No specific recommendations, but adherence to GLP principles ensures traceability of milestones and outcomes		FDA-approved methods, results are consistent with CT recommendations (if applicable)

The table is prepared by the authors

Abbreviations. *IS, internal standard; pre-CS, preclinical study; CI, confidence interval; CS, clinical study; FDA, Food and Drug Administration; GLP, Good laboratory practice; GMP, Good manufacturing practice; LLOQ, lower limit of quantification; LOD, limit of detection; PD, pharmacodynamics; QC, quality control; SD, standard deviation*

Note.

* *in-study validation criteria are based on validation results and formulated in the same way as the acceptance criteria for test sample analysis (ECE Council Resolution No. 85 of 03.11.2016 "On Procedure of Bioequivalence Studies in the EAEU"; Guidance for industry: Bioanalytical method for validation. Rockville, 2018).*

** *for heterogenous (high molecular weight) biomarkers, calibrators are usually prepared with recombinant reference material in a surrogate matrix. The analysis evaluated only relative accuracy. The term is appropriate for almost all biomarkers where the calibration material (reference standard and matrix) differs from the endogenous biomarker.*

*** *for more detailed recommendations on the design (scope) of experiments to evaluate the precision of biomarker assay validation, refer to Table 7 in* Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.

**** *when using labelled reference standard for heterogeneous (high-molecular) biomarkers, the analysis only allows to define stability of a recombinant molecule but not the stability of endogenous biomarkers.*

***** *ECE Council Resolution No. 85 of 03.11.2016 "On Procedure of Bioequivalence Studies in the EAEU"; Guidance for industry: Bioanalytical method for validation. Rockville, 2018; Guideline on bioanalytical method validation. EMEA/CHMP/ICH/172948/2019. Amsterdam, 2022.*

it is necessary to perform validation tests to an extent that confirms the compatibility of the technique with its intended use at a specific research stage. According to A. Safavi[8], it is important to constantly revisit the assay context. The answers to "What will the data be used for?", "What conclusions will be drawn from the analysis?", "Are they qualitative or quantitative?", "Will the biomarker data be used to submit to regulatory authorities or are they only used to better understand the mechanism of action?" form the basis of the COU and FFP concepts. The concept emerged in 2005–2006 [17] and underwent several changes and discussions in *ad hoc* working groups and during extensive scientific discussions at conferences, in scientific publications, etc. [18–20]; it was documented in detail in 2018–2021[9] [21]. The legal status of the document[10] entitled "Points to Consider" is not clear – in some papers, it is marked as the final document prepared by Critical Path Institute (C-Path) in USA and Food and Drug Administration (FDA) [22]. However, at the time of this writing, it was not available on the FDA website. Apparently, it is the predecessor of a regulatory guideline, and its recommendations are to be followed in practice. Notably, the concept and the document are subject to further improvement and development of approaches considering different expert opinions in this field [22–24], and the key provisions were included in the FDA document on the use of biomarkers for clinical studies of new veterinary drugs[11].

Stages and key elements of biomarker analytical validation. Biomarker analysis according to the fit-for-purpose concept (FFP) is a process that accompanies drug development using a biomarker (regardless of the role of the biomarker in this process) and includes (according to [18]) four continuous stages: methodology development and pre-validation, research methodology validation, complete or extensive methodology validation, and methodology validation in a study *(Figure 1)*. Measurements subsequently used to establish and confirm decision points in the biomarker clinical validation (qualification) should be sufficiently and rigorously validated to ensure that the analysis is sufficiently effective for application of the biomarker[12]. In this paper, we focus specifically on the analytical part – measuring methodology/procedure for a parameter.

Most studies point out two main stages or directions – biomarker analysis in the drug development and for diagnosis[13] [7, 17, 18, 22, 23]; they determine the scope of validation tests. Preliminary (screening) stages [17] are now also included (translational/partial validation, not found in earlier works [18, 19] and in *Figure 1*, but present in *Points to Consider*[14]) as well as comparison with the scope of validation tests for methods evaluating BE, PK, and TK of drug substances[15] [7, 17]. The recommendations are summarised in *Table 1*.

[8] Safavi A. Exploratory biomarker testing: to qualify or validate the assay? 2018.
[9] Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.
Guidance for industry. Biomarkers and surrogate endpoints in clinical studies to support effectiveness of new animal drugs. Rockville; 2021.
[10] Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.
[11] Guidance for industry. Biomarkers and surrogate endpoints in clinical studies to support effectiveness of new animal drugs. Rockville; 2021.
[12] Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.
[13] Ibid.
[14] Ibid.
[15] Ibid.

Table 1 is based on amended and changed materials[16] of [7, 17, 18]; however, the key document was the preferred source of classification/validation stages in the table[17], which does not include pre-validation stage of the technique. Considering pre-analytical issues and method development, this stage was mentioned in [17] and included elements such as describing reagent sources and reference standards; assessing lower limit of quantification (LLOQ) and the preliminary analytical range; method accuracy and precision (without recommended number of concentration levels and repetitions); calibration function; reagent specificity (according to supplier's information from the literature), matrix effect; arranging feasible conditions for collection, processing, storage of reagents and samples, and recommendations on the number of concentration levels and repetitions for validated diseases and bioanalytical methods, etc. This should be called a preparatory and exploratory stage that does not end up with analysing experimental samples to obtain values for decision-making, even within pre-CS. Later, the authors[18] also noted the importance of pre-analytical factors (biomaterial, interference, sampling procedure, sampling tubes and instruments, time and conditions, primary sample processing, storage and further logistics, freezing/thawing[19]) to be outlined while the technique is being developed and validated, standardised and defined for applicability (acceptability) of variations.

The early biomarker research (screening, biomarker selection, effectiveness evaluation) will only require a simple and minimally validated analysis (*Table 1*, Research validation). However, the clinical qualification of a biomarker (assessment of the therapeutic effect of the created drug, diagnostics, etc., both within pre-CS and CS) will require a confirmed analytical result. At the same time, according to different sources, the required tests may vary, and the choice of the stage/task to attribute the validated method during biomarker analysis are not complete or ambiguous, which certainly challenges development of a single consequent algorithm for analytical biomarker validation.

Analysing the combined recommendations *(Table 1)*, noteworthy is the similarity of the key indicators used to validate a bioanalytical technique, regardless of the task. Analytical validation of the biomarker in general should include an assessment of seven parameters: accuracy (precision), range of analytical measurements (including LLOQ and the upper limit of quantification, ULOQ), parallelism (minimum required dilution and dilution linearity, if applicable), precision (within and between cycles, operators, days, and reagent lots, if applicable), selectivity, specificity, and stability (under operating conditions, short-term, long-term, during freezing/thawing).

[16] Decision of EEC Council dated 03.11.2016 No. 85 Rules for conducting bioequivalence studies of medicines within Eurasian Economic Union.
Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.
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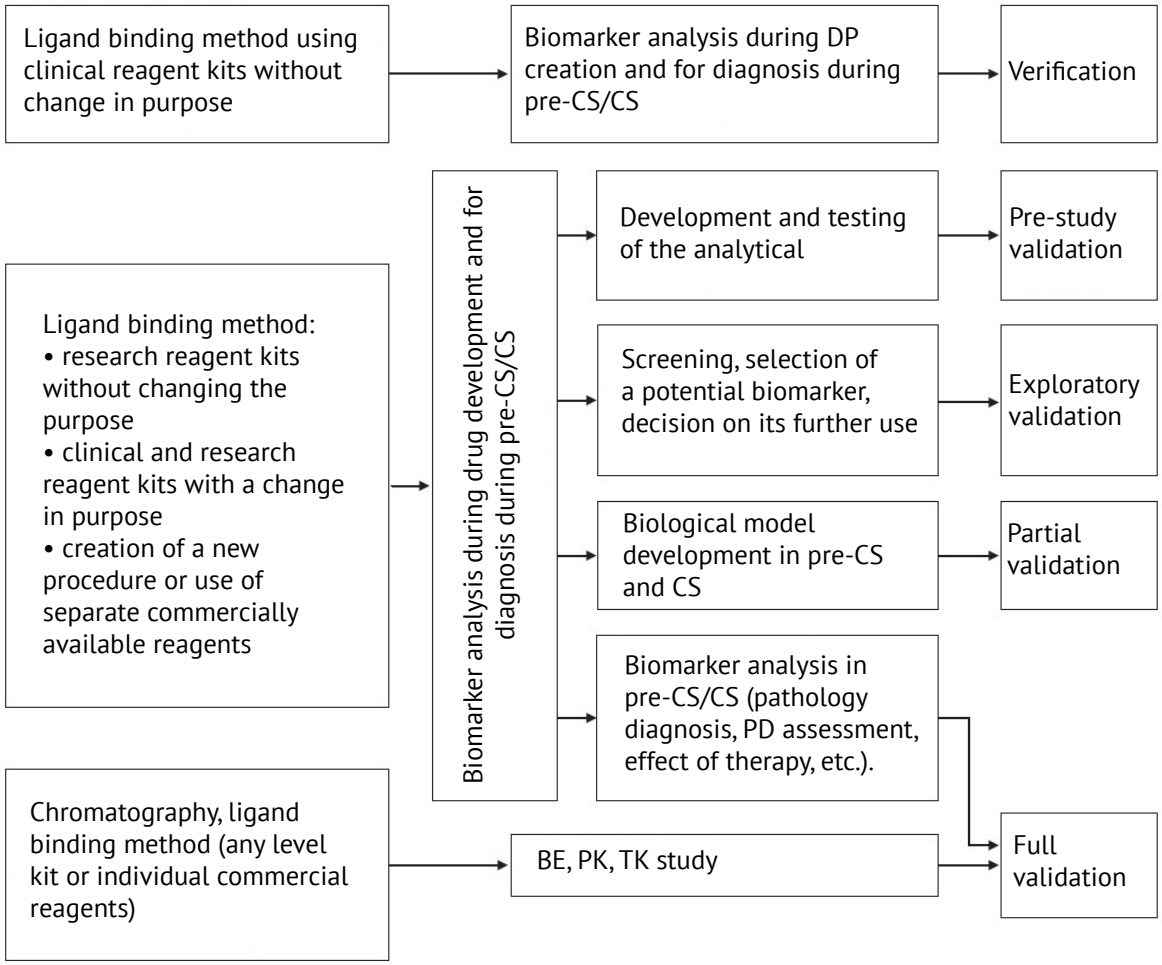
[17] Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.

[18] Ibid.

[19] Ibid.



The figure is prepared by the authors

Fig. 2. *Choosing analytical validation level of a biomarker depending on the method and the objectives; BE, bioequivalence; pre-CS, preclinical study; CS, clinical study; MP, medicinal product; TK, toxicokinetics; PD, pharmacodynamics; PK, pharmacokinetics*

Some cases require additional analytical parameters, including stability, assessment of drug interactions, etc.[20] The authors [22] emphasised that the document[21] highlighted the important assessment of parallelism and introduced the concept of an acceptable total analytical error (TAE). Notably, the concept of total analytical error sums up total error in determining the accuracy (relative systematic error, δ) and precision (CV), not exceeding 30% for ULOQ and 40% for LLOQ), and is present in the current documents regarding ligand binding methods[22]. Validation parameters, procedures, and acceptance criteria for biomarker analysis are similar, though they may not be identical to those used to obtain data on BE, FK, and TK[23]. This

[20] Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.
[21] Ibid.
[22] Decision of EEC Council dated 03.11.2016 No. 85 Rules for conducting bioequivalence studies of medicines within Eurasian Economic Union.
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[23] Guidance for industry: Bioanalytical method for validation. Rockville, 2018.

document notes that method validation for biomarker analysis should address the same issues as the validation of a method for drug analysis. ... The approach used for drug analysis should be the starting point for validating biomarker analysis, although FDA understands that some characteristics may not apply or require various considerations[24].

Opinion of A. Safavi[25], which presents differences/similarities between biomarker validation and qualification, deserves to be highlighted, apparently from the purely analytical perspective of these processes (without affecting clinical validation/qualification of the biomarker). In his opinion, both processes are aimed at proving that the test is suitable for its intended purpose but differ in the scope and reliability of the estimated parameters and the number of replicates per each parameter. The scope of qualification procedures recommended by the author is comparable to the research and/or partial validations *(Table 1)*. Notably, qualification of analytical procedure is a term hardly found in the scientific literature and regulatory documents.

Features of an analytical biomarker validation due to the used methods. When validating biomarker analysis methodology, some points to consider originate from the underlying method, depending on the measured biomarker (protein, lipid, etc.), availability of current methods, reagent kits, and measurement features (small sample volume, laboratory or field conditions, personnel qualifications, etc.), sensitivity and selectivity requirements, as well as method availability[26]. Typically, evaluation can include plate-based analysis and various detection methods (fluorescence, chemiluminescence, electrochemiluminescence, chromogenic method, mass spectrometric assessment, and relatively new acoustic detection systems[27]). According to [23], most biomarker analysis techniques are based on one of three methods:

- chromatographic techniques to be developed *de novo*;
- ligand binding techniques to be developed *de novo*;
- ligand binding techniques using commercial reagent kits.

Commercial reagent kits may be authorised for *in vitro* diagnostics, clinical laboratory testing, or for research only. Authors [25] examined some aspects of commercial kits used in the biomarker analysis. Commercial reagent kits should be tested on each analysed object (matrix) [22]. According to the authors [22], clinical laboratory kits may differentiate the need for validation and validation parameters on a case-by-case basis, depending on the intended use or characteristics of biomarkers. In this regard, it appears correct to discuss verification of methods aimed to confirm that the requirements used at its earlier validation (for example, by a manufacturer of reagent kits) are fulfilled in a specific laboratory (using a set from a certain batch and equipment available in the laboratory). These aspects seem to be similar to those discussed in [26] in relation to the activities of clinical diagnostic laboratories that perform biomedical biomarker tests as well. According to the publication based on analysis of relevant international regulatory documents, a minimum list of tests verifying clinical and laboratory studies should include intermediate precision, accuracy,

[24] Piccoli SP, Sauer JM. Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path); 2019.
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[27] Ibid.

linearity and range of calibration function, calibration verification, and confirmation of applicability for biological reference intervals specified in the manufacturer's manuals (if applicable) [26].

Research kits (intended for research only) are fully validated regardless of validation information in the operating manuals [22]. It is recommended to choose a specific kit when fully understanding whether it corresponds to the intended use of the relevant biomarker (such as calibration range or specificity). The reference standard included in a kit is evaluated using other commercially available products, paying attention to the differences from the endogenous substance, which is the target analyte. When the kit lot changes, make sure to confirm that the differences in the measured analyte concentration of the same sample are acceptable. Since research kits may become unavailable (discontinued production, disrupted economic contacts or supply), alternative analytic approaches should be considered (for example, separate use of reagents, not as part of the kit, if applicable).

Choosing the level of biomarker analytical validation depends on the method and the tasks. Pre-CS conducted *in vivo* using various animal species and *in vitro* using buffer media, cell lines, isolated organs, etc., may use clinical and research kits both specific to the measured marker, animal species or type of biological matrix, and with a different initial purpose. Kit application range beyond the manufacturer's recommendations for assessment of BE, PK, and TK requires full validation of the bioanalytical methodology, otherwise (including PD assessment) depending on the task. Thus, at the stage of biomarker selection, research level of validation is sufficient; when switching to model development using the selected biomarker, it is recommended to increase the validation level of the bioanalytical procedure to a partial one, similar to the full validation of the biomarker analysis method used to solve pre-CS/CS tasks, such as diagnosing abnormalities, assessing therapy effect, etc. *(Table 1)*. A similar scheme can be recommended in some other cases *(Figure 2)*.

Thus, only using clinical reagent kits as intended will minimise method validation procedures; in all other cases, relatively extensive validation tests will be required.

CONCLUSION

In conclusion, we would like to emphasize that extended use of biomarkers for drug development necessitates the improved methods of analytical quantification. Obviously, use of a biomarker should be preceded by a confirmation that the procedure is suitable for the planned goals, similar to a common procedure validating bioanalytical methods. The authors have compared key validation elements of the biomarker analysis and considered the level of analytical validation based on the method and the tasks. A more detailed scientific discussion of biomarker analysis validation is warranted, as there is still no joint scheme for this complex procedure. The discussion will allow using biomarkers more reliably, improving the quality of the accompanying research, and ultimately help introduce new effective and safe medicines into civil commerce.

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