Opinion Paper

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Implementing metrological traceability of C-reactive protein measurements: consensus summary from the Joint Committee for Traceability in Laboratory Medicine Workshop

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Abstract: The Joint Committee for Traceability in Laboratory Medicine (JCTLM) currently lists the secondary commutable certified reference material (CRM) ERM DA-474/IFCC (DA-474) "C-Reactive Protein in Human Serum" and two generic immunoassay-based method principles as the basis for implementing the metrological traceability of C-reactive protein (CRP) measurements by end-user measurement procedures used by medical laboratories. The current metrological traceability has produced well harmonized results for clinical samples among different end-user measurement procedures. New higher-order pure substance and secondary commutable CRMs have been nominated for listing by the JCTLM. However, the data supporting performance of these new candidate CRMs, including use of new mass spectrometry based candidate reference measurement procedures (RMPs), was not clear regarding the influence that introducing these new CRMs would have on the current well harmonized results achieved with the existing metrological traceability to DA-474. The clinically relevant CRP measurand in blood serum or plasma is a pentamer of identical subunits, which adds complexity to the application of higher-order CRMs and RMPs. The JCTLM convened a workshop in December 2022 to review the appropriate implementation of metrological traceability of CRP measurements. The workshop consensus was that the extent-of-equivalence data must include considerations about the impact of a new CRM when used for its intended purpose in the calibration hierarchies of existing end-user measuring systems; and that a new RMP must compare results with another existing well validated candidate RMP or with a globally available end-user measurement system.

Keywords: C-reactive protein; Joint Committee for Traceability in Laboratory Medicine (JCTLM); metrological traceability

The database maintained by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) currently lists the secondary commutable certified reference material (CRM) ERM DA-474/IFCC (DA-474) "C-Reactive Protein in Human Serum" and two generic immunoassay-based method principles (immunoturbidimetry and immunonephelometry) as the basis for implementing the metrological traceability of C-reactive protein (CRP) measurements. The DA-474 certification report states that its value was assigned by 6 commercial immunoassay measurement procedures that each used ERM DA-470 as calibrator [1]. The value assigned to ERM-DA-470 is traceable to the 1st International Standard for CRP from the World Health Organization, WHO 85/506, as described in the certification report. The WHO 85/506 standard was prepared in 1985 and each ampoule contains the residue after freeze drying of a solution of 50 µg human CRP in 0.5 mL pooled human serum from healthy individuals [2]. The commutability with clinical samples of primary calibrators prepared as solutions of WHO 85/506 is unknown.

The calibration hierarchies of currently available commercial end-user immunoassays are based on DA-474 secondary commutable CRM or its predecessor ERM DA-472/IFCC used as higher-order references for metrological traceability conforming to the International Standards Organization standard 17511 [3]. The CRP used to produce the ERM series of CRMs was shown to be the pentameric form [4, 5]. The current metrological traceability of CRP immunoassays has produced a clinically fit-for-purpose agreement among clinical sample results when measured by a representative number of end-user measurement procedures [6]. The two secondary commutable CRMs have maintained this consistent good performance for 15 years.

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From a metrological traceability perspective, a desirable goal is to have a calibration hierarchy that includes a primary pure substance CRM and a higher-order reference measurement procedure (RMP) for the measurand to enable consistently repeatable metrological traceability over long time intervals and in any location globally. Developing these higher-order references has been challenging for CRP because it is a complex protein that exists as a pentamer of identical subunits in its circulating form in clinical blood samples [7]. Therefore, the clinically relevant measurand is pentameric CRP in blood serum or plasma and end-user immunoassays used by medical laboratories are designed to measure preferentially the pentamer and not react with monomers.

The complexity of the CRP measurand means that developing a higher-order RMP for CRP must appropriately address the pentameric measurand to be fit for purpose in the calibration hierarchy for an immunoassay. A higher-order RMP based on mass spectrometry that digests the measurand into peptide fragments will measure a quantity (peptide) that is different than the quantity (pentameric protein) present in the clinical sample or in a secondary CRM, such as DA-474, that is commutable with clinical samples when measured using immunoassays. If the proportion of monomer is different between the calibrator used for the RMP and the sample measured by the RMP, which in this case is a secondary commutable CRM, then the measurand value assigned to the CRM will not be correct and will introduce a bias in the calibration hierarchies of commercially available immunoassays.

Consequently, the pentamer to monomer ratio of a primary pure substance CRM for CRP used to calibrate a RMP for the measurand is critical to the suitability of the primary CRM and of the candidate RMP. A primary CRM must be pentameric when its intended use is to prepare secondary commutable calibrators for immunoassays. Otherwise, the primary CRM's assigned value will not correctly reflect the pentameric form. In addition, the pentamer structure must be preserved when primary calibrators are prepared from the primary CRM. Therefore, the full characterization of a primary CRM and its use to prepare primary calibrators for implementing traceability of CRP measurements must be unambiguously described for demonstrating that it is suitable and appropriate for its intended use.

The JCTLM requires demonstration of extent-of-equivalence to an existing CRM or RMP when a new candidate CRM or RMP is nominated for listing in its database [8, 9]. In the case of CRP, there is currently no primary CRM or RMP listed for the measurand. In this situation, the JCTLM policy in effect at the time of the workshop held in Milan in December 2022 recommended a comparison study for a new candidate RMP to be conducted with existing measurement procedures used by medical laboratories in order to check similarity in measurand selectivity. The JCTLM policy did not specify a specific procedure how to conduct this comparison for a new candidate primary CRM when there is not an existing one listed. However, for a complex measurand such as CRP, the influence of monomeric forms must be considered a priority.

Data were shared at the workshop by two National Metrology Institutes who have developed candidate primary pure substance and secondary CRMs, and a mass spectrometry-based candidate RMP for CRP. Some of these CRMs had been nominated for review by the JCTLM. Based on the data shown, results for clinical samples measured with procedures calibrated with these nominated CRMs may significantly differ from results when measured using currently available well harmonized end-user immunoassays calibrated to DA-474 or DA-472. This situation presented a challenge for the JCTLM review process as the potential bias may cause erroneous interpretation of patient results.

The data shown at the workshop demonstrated the risk that introducing new candidate higher order references and therefore defining new calibration hierarchies for end-user immunoassays could cause disagreement for clinical sample results when compared to results based on the existing metrological traceability to WHO 85/506. Medical laboratory practice would be negatively impacted by JCTLM listing a new CRM or higher-order RMP that introduced a bias in an existing well established calibration hierarchy. The workshop concluded that information supporting nomination of a new CRM or higher-order RMP for CRP must include assessment of the influence of these new candidate references on the results for clinical samples measured using end-user measurement procedures. The same concept also applies to metrological traceability for other measurands if a good harmonization status is already in place.

Following the workshop, the JCTLM policies for extent-of-equivalence experiments were updated to state the requirements more explicitly. For CRMs, clause 6.2 of the JCTLM-DBWG-P-04A procedure now states "The extent-of-equivalence of materials must include considerations about the impact of a new CRM when used for its intended purpose in the calibration hierarchies of existing end-user measuring systems." [8] For RMPs, clause 6.1.2 of the JCTLM-DBWG-P-04B procedure now states "In the case where no higher-order RMM/Ps are listed by JCTLM to allow for such extent-of-equivalence studies to be performed, the RMM/P nominator is required to conduct a comparison study of its nominated RMM/P with at least one existing well validated candidate RMM/P or globally available end-user measurement procedure used by a routine measurement laboratory." [9].

In summary, the workshop consensus was driven by the general belief that producers and users of CRMs and RMPs

have a common goal and shared responsibility to ensure that metrological traceability of medical laboratory end-user measurement procedures produces fit-for-purpose results to meet the medical care needs of patients around the world. To this end, the JCTLM will continue to collaborate with all stakeholders to promote best practices for metrological traceability implementation and support.

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