

ERM[®]-DA483/IFCC

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Use of ERM- DA483/IFCC for the Quantification of *Immunoglobulin G proteinase 3 anti-neutrophil cytoplasmic autoantibodies*

The certified reference material (CRM) ERM-DA483/IFCC [1] is intended to be used for the calibration of immunoassay-based *in vitro* diagnostic devices.

INTRODUCTION

ERM-DA483/IFCC is a serum protein reference material intended for the standardisation of measurements of Immunoglobulin G proteinase 3 anti-neutrophil cytoplasmic autoantibodies (IgG PR3 ANCA).

These are antibodies against the neutrophil enzyme proteinase 3. They are detected as anti-neutrophil cytoplasmic antibodies and represent the cornerstones of the diagnosis of small vessel associated vasculitis. IgG PR3 ANCA are found in about 80 % of patients with granulomatosis with polyangiitis (GPA), and also in about 35 % of patients with microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA), and renal-limited rapidly progressive glomerulonephritis [1, 2].

The marked diversity in the response of methods available for analysis and the materials used for assay calibration, mandate for a certified reference material (CRM).

The EU Directive on *In Vitro* Diagnostic Medical Devices (IVD-MD) (Directive 98/79/EC) requires traceability of calibrants and control materials to reference measurement procedures and/or reference materials of higher order.

A CRM is required to have an assigned value that is metrologically traceable, and accompanied by an uncertainty statement. The stability and homogeneity with respect to the certified property must be verified, and

the CRM must be commutable [3]. These attributes are particularly challenging for serum protein calibrants, as they form a mixture of interacting proteins with different isoforms and complexes.

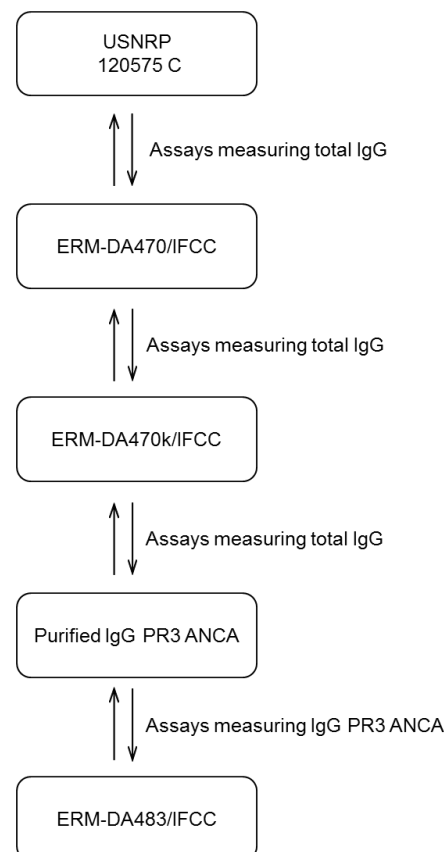


Figure 1: Reference materials and methods used for establishing the metrologically traceability chain (arrows facing upwards) and calibration sequence (arrows facing downwards)

INTENDED USE OF ERM-DA483/IFCC

ERM-DA483/IFCC is intended for the calibration of immunoassay based *in vitro* diagnostic devices or control products quantifying the IgG PR3 ANCA content of a patient serum sample. The material and dilutions thereof have shown to be commutable for a number of immunoassays using different reagents and platforms. Commutability of a material is of the utmost importance as it defines its fitness for use. A material is commutable when its analytical behaviour is highly equivalent to the behaviour of patient samples [2]. The IgG PR3 ANCA methods that have been tested and for which the CRM is commutable are listed in the Annex of the certification report [4].

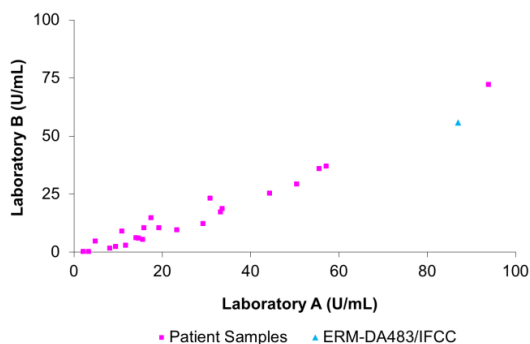


Figure 2: Example of commutability testing for two laboratories.

Limitations

If ERM-DA483/IFCC is used with methods for which commutability has not been assessed the user should ascertain that the CRM is suitable.

PRACTICAL USE OF ERM-DA483/IFCC

ERM-DA483/IFCC is provided in lyophilised form. The material has thus to be reconstituted with ultra-pure water one day before use. The reconstitution protocol includes a number of steps that need to be followed:

- Remove the vial from the freezer and place it in the room where the balance is located one hour before reconstitution.
- Prior to reconstitution, tap the bottom of the vial gently on the surface of the

table. Make sure that all the material has settled down on the bottom of the vial. Remove the screw cap.

- Weigh the vial together with the rubber stopper. Note the mass or press the "TARE" button on the balance. Lift carefully the rubber stopper until the groove.
- Add 1.00 mL of ultra-pure water through the groove, and press the rubber stopper back into place. Weigh the vial and note the mass. If you have used the "TARE" function, the value can be used directly for the mass m . Otherwise the first mass must be subtracted from the second to obtain m .
- The concentration of a particular protein in the solution, corrected for the reconstitution mass, can be obtained by multiplying the certified value for that protein with intended / m , with intended the mass intended to be added (1.0000 g).
- Leave the vial at room temperature for one hour, then gently mix by inversion at least five times (do not shake it) during the next hour.
- Leave the vial at room temperature overnight. The following day, gently mix by inversion five times in a period of one hour, prior to starting the analysis.

Table 1: Certified mass concentration and calculated expanded Uncertainty

	Mass Concentration	
	Certified value ²⁾ [mg/L]	Uncertainty ³⁾ [mg/L]
IgG PR3 ANCA ¹⁾	270	29
<p>1) proteinase 3 anti-neutrophil cytoplasmic antibodies as measured by immunoassays</p> <p>2) Unweighted mean value of the means of 10 accepted data sets each set obtained in a different laboratory and/or with a different method of determination. The certified mass concentration and its uncertainty are traceable to the stated value of the mass concentration in United States National Reference Preparation (USNRP) 12-0575C [5]</p> <p>3) The uncertainty is the expanded uncertainty of the certified value with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008</p>		

UNCERTAINTIES

The uncertainty associated with the certified mass concentration of the ERM-DA483/IFCC must be taken into account when calculating and/or reporting the uncertainty of the mass concentration of IgG PR3 ANCA present in a patient sample.

HANDLING OF THE MATERIAL

The lyophilised material should be stored at -70 °C or -20 °C until use. After reconstitution the material can be stored at 4 °C for up to one week.

The certified value of the mass concentration of IgG PR3 ANCA and its associated uncertainty are only valid when the minimum sample intake specified on the certificate of 5 µL is respected.

REFERENCES

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3. ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement, (GUM 1995), International Organization for Standardization, Geneva, Switzerland, 2008.
4. E. Monogioudi, D. P. Hutu, J. Charoud-Got, J. Sheldon, H. Schimmel, S. Trapmann, P. L. Meroni, H. Emons and I. Zegers, Certification report, The certification of immunoglobulin G proteinase 3 anti-neutrophil cytoplasmic autoantibodies (IgG PR3 ANCA) in human serum ERM®-DA483/IFCC, European Commission. Joint Research Centre, Institute of Reference Materials and Measurements, EUR 28537 EN–2017.
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