A WHO Reference Reagent for lupus (anti-dsDNA) antibodies: international collaborative study to evaluate a candidate preparation

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ABSTRACT

Introduction Antibodies against double-stranded DNA (anti-dsDNA) are a specific biomarker for systemic lupus erythematosus (SLE). The first WHO International Standard (IS) for anti-dsDNA (established in 1985), which was used to assign units to diagnostic tests, was exhausted over a decade ago.

Methods Plasma from a patient with SLE was first evaluated in 42 European laboratories. The plasma was thereafter used by the National Institute for Biological Standards and Control to prepare a candidate WHO reference preparation for lupus (anti-dsDNA) antibodies. That preparation, coded 15/174, was subjected to an international collaborative study, including 36 laboratories from 17 countries.

Results The plasma mainly contained anti-dsDNA, other anti-chromatin antibodies and anti-Ku. The international collaborative study showed that the field would benefit from 15/174 as a common reference reagent improving differences in performance between different assays. However, no statistically meaningful overall potency or assay parallelism and commutability could be shown.

Conclusion 15/174 cannot be considered equivalent to the first IS for anti-dsDNA (Wo/80) and was established as a WHO Reference Reagent for lupus (oligo-specific) anti-dsDNA antibodies with a nominal value of 100 units/ampoule. This preparation is intended to be used to align test methods quantifying levels of anti-dsDNA antibodies.

INTRODUCTION

Antibodies against double-stranded DNA (anti-dsDNA) are biomarkers for systemic lupus erythematosus (SLE).1 2 As a result, measurement of anti-dsDNA is widely used as a diagnostic and prognostic test for SLE, and there are a range of kits and tests available.3

The first WHO International Standard (IS) for anti-dsDNA denoted Wo/80 was established in 1985 to assign International Units (IU) to diagnostic tests.4 Wo/80 was exhausted over a decade ago and requires replacement.

MATERIALS AND METHODS

Raw material characterisation by 42 European laboratories

Plasmapheresis plasma from a female patient with SLE diagnosed according to the 1997 classification criteria1 6 was evaluated blindly by 42 European laboratories in the European Consensus Finding Study Group on autoantibodies, a.k.a. the EULAR Autoantibody Study Group (https://www.eular.org/investigative_rheumatology_study_groups.cfm). Sampling was approved by the local Institutional Review Board, and the patient had given written informed consent.

Treatment of the candidate standard (15/174) and commutability samples (S1–S3)

2.4 mL of plasma, evaluated above, and three patient samples with SLE coded 15/174, S1, S2 and S3, respectively, were thrombin-treated,7 clarified, 0.5 mL filled into glass ampoules and lyophilised at the National Institute for Biological Standards and Control (NIBSC).7 8 Accelerated stability studies on 15/174 showed a reduction in activity of 0.09%/year at −20°C (online supplementary table S1).8

Participants and assays used in the international collaborative study

In an international collaborative study (36 laboratories from 17 countries; online supplementary table S2), 15/174 was compared with local standards and S1–S3 to evaluate commutability. Each laboratory is referred to by an arbitrarily assigned number (1–36). For a laboratory performing more than one method, each method is treated as if performed by separate laboratories. A total of 26 different methods were used (table 1). Participants were requested to contribute full dilution comparisons and content estimates of 15/174 with local
standards, and assay replicate dilution series in duplicate. Three independent assays were requested, on separate days, with dilutions from freshly reconstituted ampoules.

Study participants were recruited separately to the European and international studies and both were run independently of each other.

Statistical analyses
At NIBSC, estimates in IU/mL at each sample dilution as reported by participants were used directly in the analysis to calculate the potency of 15/174 and S1–S3. A geometric mean (GM) of 15/174 and S1–S3 for each laboratory. GM was used to combine results from individual assays.

Patient involvement
There was no patient involvement in this study.

RESULTS
Characterisation of the raw material by 42 European laboratories
Homogeneous antinuclear antibody or AC-1 pattern was detected in all laboratories (figure 1A). All laboratories reported anti-dsDNA (figure 1B). Anti-histone antibodies were reported from 24/25, anti-nucleosome in 18/19 and anti-Ku by 18/18 laboratories. Other autoantibodies were rarely reported (online supplementary table S3).

Assay validity for the international collaborative study
Individual assay estimates and instances of non-parallelism (with commercial kit standards or 15/174) are shown in online supplementary table S4 (calculated from reported results) and online supplementary table S5 (calculated relative to 15/174 by parallel line analysis). A summary of the extent of non-parallelism is shown in table 2.

Statistically valid estimates for 15/174 in terms of commercial kit standards or for S1–S3 in terms of kit standards or relative to 15/174 tended to be highly variable. Estimates of 15/174 against kit standards, for example, ranged from 56 IU/mL to a high of 847 IU/mL, although most estimates fell in the 100–200 IU/mL range (figure 1C). Similar variability for S1–S3 was also observed (online supplementary figure S1A-J). Endpoint titres for the C. luciliae immunofluorescence test (CLIFT) were also highly variable, ranging from 50 to 1000 for 15/174 (figure 1D and online supplementary figure S2A-C).

Intra-laboratory and inter-laboratory variability for the international collaborative study
Calculation of estimates in terms of a common candidate standard invariably produced a reduction in %GCV, most noticeable for CLIFT results when comparing the estimates in terms of absolute titres with those obtained relative to 15/174 (table 2).

For quantitative immunoassays, comparing estimates reported in terms of kit standards against those calculated in terms of 15/174 showed reductions in %GCV for S1&S2, but not S3, based on results after exclusion of outliers (table 2).

DISCUSSION
In all laboratories and test methods, 15/174 exhibited anti-dsDNA reactivity. In approximately half of the laboratories, the material behaved in an apparently similar way to local standards, and by inference to the first IS (Wo/80). In a similar number of laboratories, there was observable non-parallelism and no quantitative traceability to Wo/80 could be established. Moreover, across the entire study, it was not possible to establish commutability, as a consistent ranking order for the three patient samples was not obtained. In this context, it is important to mention that
anti-dsDNA levels in S1 and S2 were very similar (table 2). It is also of interest to note that laboratories 18.2, 18.3 and 21 used high sensitivity addressable laser bead immunoassay kits and the higher potency results reported were probably due to detection of low affinity antibodies (figure 1C). However, as shown in table 2, alignment to 15/174 reduced variability and minimised batch-to-batch variability of a test kit, rather than obtaining an absolute value, should be considered most important in clinical diagnosis and monitoring disease progression and/or the effect of treatment over time.

The range of available anti-dsDNA assays has increased considerably during the 30 years since Wo/80 was described. The 36 laboratories participating in the collaborative study for 15/174 all employed the assays routinely used to quantify anti-dsDNA in their routine clinical settings. This setting differs appreciably from the setup employed in the evaluation of Wo/80 when eight laboratories with special expertise in anti-dsDNA testing all obtained identical centrally prepared reagents and then performed CLIFT and Farr assays according to fixed protocols.

The first IS for anti-dsDNA, Wo/80, is exhausted and has been unavailable for more than a decade. Some methods included in this study can provide a historical linkage to the unitage assigned to Wo/80 through earlier calibration exercises, although many cannot, and none can be confirmed due to the unavailability of Wo/80. Thus, the typical replacement paradigm is not applicable when introducing 15/174. Another problem in exchanging autoantibody standards based on patient’s samples is that the candidate replacement is unlikely to be identical to the original. This is particularly significant in the case of anti-dsDNA where autoantibody avidity might differ between patients. SLE sera also contain many different autoantibodies against a range of systems.
antigens, including dsDNA. Although 15/174 cannot be regarded as monospecific for anti-dsDNA, we found the raw material for 15/174 to be rather specific for anti-chromatin reactivities and antibodies against Ku, a DNA binding protein.\textsuperscript{12-14} It is known that different methods may detect different populations of anti-dsDNA\textsuperscript{1} so there are discrepancies in antibody levels depending on the type of test when applied to individual patients.

Given the presumed different binding characteristics resulting in an apparent lack of comparability of this candidate standard with the previous IS, it is considered that it would be unwise to establish this material as a replacement IS, with a defined unitage in IU. Notwithstanding this discontinuity, this study showed that the field would benefit from the availability of an international reference reagent, and that the current situation, with manifest differences in performance between different assays supposedly measuring the same thing, would be improved. The preparation 15/174 is intended to be used to align test methods quantifying levels of anti-dsDNA to a common standard.

Based on the results presented here, the WHO Expert Committee on Biological Standardisation endorsed preparation 15/174 as the WHO Reference Reagent for lupus (oligospecific) anti-dsDNA antibodies with a nominal potency of 100 units per ampoule.\textsuperscript{15} The name intentionally emphasises the non-continuity with the first IS for anti-dsDNA. The Reference Reagent 15/174 is available from the NIBSC (https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=15/174).

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Contributors PLM, JR, CD and BJF evaluated the suitability of the proposed candidate material; JH and PR carried out the statistical analysis; BJF and JR drafted the manuscript, which was critically reviewed and approved by all the authors.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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