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# An international collaborative study to establish the WHO 1st international standards for C1-inhibitor, plasma and concentrate

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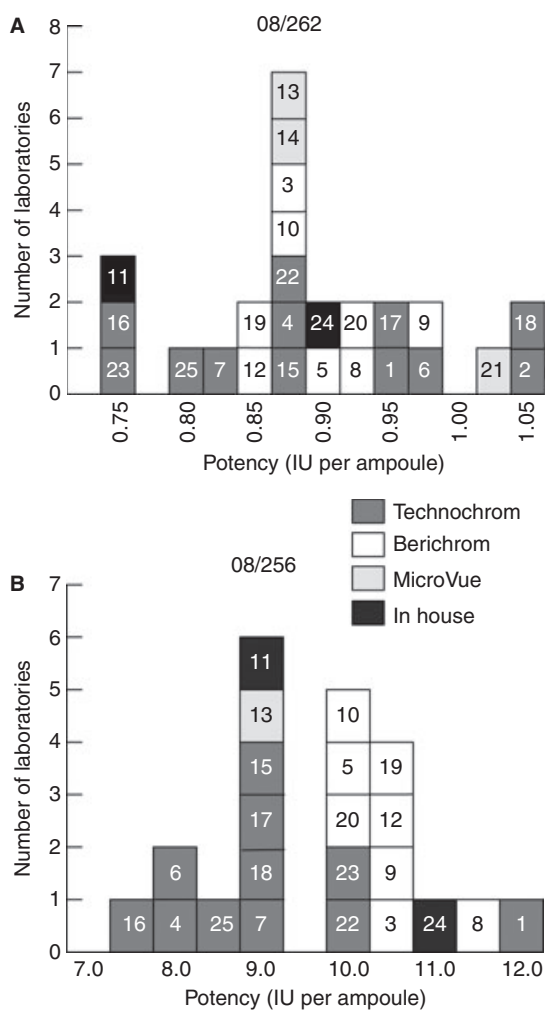
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C1-inhibitor (C1-esterase inhibitor, C1-inh, C1-INH) is a member of the plasma serpin (serine protease inhibitor) family involved in the regulation of both complement and contact system activation. C1-inh deficiency is most commonly associated with a life-threatening swelling disorder, hereditary angioedema (HAE), which presents as recurrent attacks of edema typically affecting the face, mouth and/or upper airways. Patients with HAE lack functional C1-inh, which may be treated with C1-inh replacement therapy. Diagnostic tests for functional C1-inh in patient plasma and for C1-inh potency of therapeutic products is determined using a chromogenic or an ELISA assay to measure complex formation of C1-inh with C1-esterase. An investigation into the uniformity of C1-inh measurement throughout Europe was undertaken [1] and among the recommendations from this study was development of an international standard (IS) for C1-inh to reduce inter-laboratory variation and support harmonization of international regulations. A project to calibrate two new WHO international standards for C1-inh, a plasma standard for the diagnosis of C1-inh deficiency and a concentrate standard for potency labelling of therapeutic products, was endorsed by the Expert Committee on Biological Standardisation (ECBS) of the World Health Organization (WHO) in October 2007. Two candidate materials were filled, a plasma candidate coded 08/262 (sample A) using normal human plasma sourced from the UK Blood Authority (North London Transfusion Centre, Colindale) and a concentrate candidate coded 08/256 (sample B) using a plasma-derived therapeutic product donated by a manufacturer. The materials were filled and freeze-dried in accordance with the conditions required for IS [2]. Further details of the candidate material fills and an

assessment of stability are provided in Data S1. An international collaborative study was organized, with 28 participating laboratories from 13 different countries. Each laboratory was provided with six ampoules of each sample (A and B) and was asked to prepare a normal plasma pool (N). Ideally N was collected fresh on the day of the assay; however, use of frozen plasma was accepted. The aim of the study was to assign potency values to samples A and B relative to N, which was assigned a nominal potency of 1.0 IU/mL, consistent with the existing unit definition. Four independent C1-inh assays were requested, including a minimum of three dilutions for each sample in duplicate. Most laboratories used a chromogenic assay, either an in-house method or one of the two commercial kits: Berichrom C1-inhibitor (Siemens Healthcare Diagnostics, Marburg, Germany) or Technochrom C1-INH (Technoclone, Vienna, Austria). Several laboratories used the MicroVue C1-Inhibitor Enzyme Immunoassay (Quidel Corporation, San Diego, CA, USA). The test procedures provided with commercial kits describe a method for measuring the C1-inh content of human plasma at a single dose; however, a full statistical analysis of the results requires multiple doses with replication. A modified test procedure was developed at the National Institute for Biological Standards and Control (NIBSC) for the Technochrom and Berichrom assay kits that allowed multiple C1-inh dilutions to be assayed, and these protocols were available to participants if required. Laboratories that already performed multiple dose assays were encouraged to use their own method wherever possible. Raw data were returned to NIBSC for analysis; chromogenic results were analysed using a slope ratio model and immunoassay results were analysed using a parallel line model. Potencies and confidence limits for samples A and B were calculated relative to N for each assay using the software program COMBISTATS [3] and validity criteria were applied according to the European Pharmacopoeia [4]. The potency result for each sample was taken as the geometric mean of all laboratory means, including all valid assays, with the geometric coefficient of variation (GCV) representing the inter-laboratory variation. The

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potency of the plasma candidate (sample A) was calculated from the results from 100 independent assays performed by 25 laboratories, with an overall mean of 0.89 IU (GCV = 9.9%). The potency for the concentrate candidate (sample B) was calculated from 88 independent assays from 22 laboratories, with an overall mean of 9.6 IU (GCV = 12.8%). Results are presented in Tables S1 and S2, and Figures S1 and S2. Of the 25 laboratories that contributed results to the final calculation, 9 used the Berichrom kit, 11 used the Technochrom kit, three used the MicroVue kit and two used in-house methods. The distribution of results for both samples is shown in Fig. 1, which also indicates the assay method used. For sample A, freeze-dried pooled plasma against fresh plasma pool N, the results appear to be distributed randomly, with no observed bias for any particular method. For Sample B, freeze dried C1-inh concentrate against normal plasma pool N, the overall distribution of results is acceptable; however, there is a



**Fig. 1.** Distribution of results for C1-inh potency of candidate A (08/262) and B (08/256). Columns represent the number of laboratories with results in the corresponding C1-inh concentration range. Each box represents the mean result for an individual laboratory; the number identifies the laboratory code, and the shading represents the assay method used. The mean potency value assigned to the plasma candidate 08/262 was 0.89 IU and mean potency of the concentrate candidate 08/256 was 9.6 IU.

significant trend for the Berichrom kit to produce higher potency estimates, and the Technochrom kit to produce lower potencies. This difference is likely to be a result of matrix effects caused by other components in the normal plasma pool N, and highlights the importance of establishing a separate concentrate IS, to eliminate the need to measure concentrates against plasma in the future. With a concentrate, rather than plasma, IS we would expect potency assignment of purified concentrates to become independent of the method, which should reduce inter-laboratory variation and harmonize the potencies of current and future products. Future replacement IS will be calibrated against the current IS with a check against normal plasma, in line with current practise. Preparation 08/262 was proposed as the WHO 1st IS for C1-inh, plasma, with a potency of 0.89 IU, and preparation 08/256 was proposed as the WHO 1st IS for C1-inh, concentrate, with a potency of 9.6 IU. The proposals were agreed to by the collaborative study participants, and by a panel of experienced scientists selected by the chair of the ISTH-SSC subcommittee on Factor XI and the contact system, and two new international standards were established by the ECBS of WHO in 2010. Long-term stability studies are ongoing and results to date indicate that both materials are very stable, with no observed loss of potency after 1 year of the 08/262 up to +20 °C, or of 08/256 up to +56 °C. It was noted by the ECBS that the new concentrate standard has not yet been evaluated for the potency estimation of recombinant products and this is reflected in the 'Instructions for Use' for 08/256.

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### Disclosure of Conflict of Interest

The authors state that they have no conflict of interest.

### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Candidate material fills.

**Figure S1.** Mean C1-inhibitor potencies of sample A (IU per ampoule) for each laboratory relative to local normal plasma pools.

**Figure S2.** Mean C1-inhibitor potencies of sample B (IU per ampoule) for each laboratory relative to local normal plasma pools.

**Table S1.** Mean C1-inhibitor potencies of plasma sample A (IU per ampoule) for each laboratory relative to local normal plasma pools.

**Table S2.** Mean C1-inhibitor potencies of sample B (IU per ampoule) for each laboratory relative to local normal plasma pools.

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