Automated Dibucaine Number Measurement with DuPont Dimension® ES and AR Analyzers

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We report a fully automated method for determining dibucaine number (DN) in a single-run procedure involving Dimension® cholinesterase (CHE) Flex™ pseudocholinesterase (P)CHE reagents. The method was developed and optimized with the "open channel" and "kinetic" software facilities of the Dimension-ES instrument, where the DN is calculated automatically by an algorithm from the ratio of the uninhibited and inhibited rates, measured bichromatically, from a single analysis. The protocol was satisfactorily assessed for substrate depletion, linearity, reagent stability, and the effects of different dibucaine concentrations. Validation was performed across a range of CHE activities (1.5–22 kU/L) representing the three main genotypes, UU, UA, and AA. The respective DNs (mean ± SD), determined on the Dimension-ES, were 82.0 ± 1.6 (n = 32), 71.0 ± 3.1 (n = 10), and 23.0 ± 2.7 (n = 14), with corresponding imprecisions (CV) of 0.3%, 0.6%, and 5.2% (intraassay) and 0.7%, 0.7%, and 8.6% (interassay). Comparisons with reference (x) laboratory values and the DuPont aca® (x') procedure (n = 53) gave regression equations of: y = 0.88x + 11.2, r = 0.99, and y = 0.85x' + 11.9, r = 0.99. A separate trial conducted with a Dimension-AR instrument gave similar performance. We conclude that the new DN method is fast, efficient, and appropriate for clinical use.

Indexing Terms: cholinesterase/anesthesiology/intermethod comparison/genetic disorder/enzyme activity/bichromatic analysis

Atypical forms of cholinesterase [CHE; EC 3.1.1.8; also known as pseudocholinesterase (PCHE)] can be detected by measurement of the dibucaine number (DN). The sensitivity of CHE to dibucaine inhibition is used to distinguish between congenital and acquired forms of abnormal CHE activity and to assign genotype to patients (1, 2).

CHE rapidly hydrolyzes succinylcholine (suxamethonium), a muscle relaxant commonly used to provide short-term relaxation, primarily to facilitate intubation of the trachea following the induction of general anesthesia and before the beginning of surgical procedures (3). It is also frequently used to ablate motor activity that occurs during electroconvulsive therapy (3, 4). Inherited forms of abnormal CHE are present in ~4% of the general population. Only those who are homozygous for the atypical variant, however, will have significant prolongation of muscle paralysis and apnea, the duration (10 min–4 h) depending on the precise genotype of the patient (1, 5). This phenomenon occurs in <1 in 1500 humans (1). The determination of DN is superior to measurement of CHE activity alone for discriminating between genotypes (1).

Current methods are based on deriving a DN from inhibited and uninhibited CHE activities, thus requiring two separate analyses (6–9). Using the "open channels" and "kinetics" software facilities on the DuPont Dimension® ES analyzer, we developed a fast, single-run DN method. This was subsequently automated by means of a software conversion to a flexible channel, which allowed the method to be implemented as part of the normal European test panel available on the instrument. The determination of the DN is based on an existing assay of CHE activity, in which CHE hydrolysis of the substrate butryrylcholine directly reduces a blue dye, 2,6-dichlorophenol-indophenol, to its colorless derivative (10).

Method validations were performed at the London Hospital Medical College and at a hospital-based clinical center in Torino, Italy.

Materials and Methods

Instrumentation and Software

The Dimension-ES and aca® clinical analyzers were obtained from DuPont UK Diagnostics and Biotechnology Systems (Stevenage, Herts, UK). At the Italian site the Dimension-AR and Hitachi 717 (Boehringer Mannheim, Mannheim, Germany) automated analyzers were used.

The Dimension "open channels" software (versions ES 1.3 and AR 2.1) was used as a developmental tool in the manipulation and definition of reaction conditions. The "kinetics" software option allowed continuous monitoring (at 5-s intervals) of the entire progress of a reaction at as many as 10 different wavelengths and automatic rate calculations at any chosen interval. Details of these procedures are described in the instrument operating instructions available on request from DuPont.

Reagents

CHE reagents for the Dimension and aca in the respective forms of "Flexes™" and "analytical packs" were obtained from DuPont UK Diagnostics and Biotechnology Systems and used within recommended...
dates of expiry. The CHE (PCHE) method for the Dimension and aca were correctly verified according to the instructions of the manufacturer. Dibucaine-HCl (cat. no. D0638) was purchased from Sigma Chemical Co. (Poole, Dorset, UK). All water used for reconstitution was DuPont purified diluent (cat. no. 710615901).

Precinorm U* and Precipath U* control sera were obtained from Boehringer Mannheim, and Liquichek control sera 1 and 2 were obtained from Bio-Rad (Hemel Hempstead, Herts, UK). Reagents used for the Hitachi 717 were purchased from Boehringer Mannheim, DN test 1040707 (/).

DN Method

The developed method included a single-cuvette analysis of the degree of dibucaine inhibition of CHE, requiring the removal of two reagent tablets from the final well of the Flex and their replacement with a 1-mL solution of 2 mmol/L dibucaine-HCl (final reaction concentration 40 μmol/L). Sample (2 μL) plus buffer, substrate, and color reagent were incubated and a two-point rate measurement was taken bichromatically at 600 and 700 nm. The dibucaine solution was then added and the inhibited rate determined from a further two-point measurement. The DN was calculated automatically by an algorithm from the ratio of the uninhibited (UR) and inhibited rates (IR), taking into account the reaction volume change after dibucaine addition:

\[ \text{DN} = \left[ 1 - \frac{\text{IR}}{\text{UR} \times \left( \frac{\text{volume}_{\text{UR}}}{\text{volume}_{\text{IR}}} \right)} \right] \times 100 \]

With samples containing a range of CHE activities (1.5–22.0 kU/L), representing the three main genotypes (UU, UA, and AA), the protocol was optimized for substrate depletion, linearity of response, CHE reagents and dibucaine concentration, timings of reagent additions, and timings of photometric readings. The DNs found corresponded to clinically appropriate values for each genotype group. Full customer details of the test conditions are shown on the DN insert sheets, available on request from DuPont.

The DN was also assessed by a method recommended by DuPont (7) for use on the DuPont aca clinical analyzer. This consisted of manually calculating the DN from a CHE uninhibited reaction and from a reaction in which the sample had been pretreated with an equal volume of 65 mmol/L dibucaine. Further details are available on request from DuPont.

Samples and Validation

Validation of the automated DN method in the UK was performed by duplicate analyses for DN and CHE activities in 53 clinical samples of defined genotype, kindly supplied by J. Britton (Dept. of Anaesthetics, Royal Postgraduate Medical School, London), who also provided corresponding DN results as measured by a reference method (6). Samples were also measured by the established two-sample aca method. Accuracy was assessed by comparisons with other methods by using scattergrams and simple linear regression. Imprecisions were assessed by the intra- and interassay variation on each of the genotype groups (n = 10 replicates or n = 10 different days for duplicate measurement, respectively).

The Italian trial of the DN method consisted of assessing intraassay precision in n = 20 replicates on three selected patients, and interassay precision on the four commercially available control sera (Precinorm U, Precipath U, Liquichek 1 and 2) over a period of 20 days. Reagent stabilities were assessed for two samples of internal quality-control human sera during 96 h after reconstitution of reagents. The developed DN method was compared with the Hitachi method in duplicate measurements of 49 clinical samples, reflecting the standard population mix of specimens tested by the laboratory. Information on the genotypes of these patients was unavailable.

Results

For the UK study, the DNs and CHE activities for each genotype and method are summarized in Table 1. Comparisons between methods are also shown as scattergrams in Fig. 1. Imprecisions (mean ± SD, n = 10) of the new DN method for AA, UA, and UU genotypes were, respectively, 23 ± 1.97, 72 ± 0.43, and 82 ± 0.25 intraassay and 23 ± 1.98, 72 ± 0.50, and 82 ± 0.57 interassay.

Intraassay precisions assessed on the three selected patient samples for the Italian trial were 87.3 ± 0.9, 70.1 ± 1.19, and 23.2 ± 0.59, whereas interassay precisions were 80.2 ± 2.03, 81.1 ± 1.97, 80.8 ± 1.27, and 81.9 ± 1.2, respectively, for Precinorm U, Precipath U, and Liquichek 1 and 2. There was no significant variation in results over a 96-h period, in which daily measurements of DN in two internal quality-control samples gave the following results (mean ± SD and CV): 75.2 ± 0.76 (1.01%) and 81.1 ± 0.69 (0.85%). Measurements of DN in the 49 clinical samples by both methods indicated the presence of two distinct groups, equivalent to UU genotypes (n = 45) and UA genotypes (n = 4). DN results for the Dimension-AR were UU = 85 ± 2.04 and UA = 73 ± 2.85; for the Hitachi they were UU = 80 ± 0.96 and UA = 68 ± 1.9.

The linearity of the curves within the specified timings of photometer readings, with or without dibucaine inhibition (Fig. 2), were sufficient for precise and reproducible measurement of the DN.

<table>
<thead>
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<th>Table 1. DN (mean ± SD) and CHE activity results.</th>
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<tr>
<td>Genotype</td>
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<td>Ref. method (6)</td>
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<td>Ref. method (7)</td>
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<tr>
<td>New DN</td>
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<td>CHE, kU/L*</td>
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* Determined with the Dimension CHE assay.

* Prevalent (normal) genotype.

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Fig. 1. Progress curves of CHE activity with and without dibucaine inhibition.

Measurements were done with the Dimension-ES "kinetics" program by the decrease in absorbance of dichlorophenol-indophenol (DIP) at 600 nm, with subtraction of the reference absorbance at 700 nm. Arrowed box numbers define additions of reactants as follows: 1, DIP; 2, sample; 3, butylnicoline; 4, dibucaine (inhibited rate) or water (uninhibited rate).

Discussion

Our developed method provided satisfactory calculation of DN over the clinically relevant range of CHE activities (1.5–22.0 kU/L) for samples of previously defined genotype. For this purpose a working concentration of 2 mmol/L dibucaine was sufficient. In the genotype groups in which the DNs are closest together, the interassay precision for UU (DN ~82) and UA (DN ~72) genotypes was ~<2.5%, similar if not superior to that of established two-sample methods (6–9). This is more than adequate to permit discrimination between these genotypes, even allowing for the ~<5% CV scatter of DN within each genotype group. The possibility of any overlap in DN between the groups is therefore negligible, precluding the need for any further statistical treatment. Although the DN precision for the AA genotype is lower (at ~8.5%) than the precision for the other groups, the DNs of ~23 will obviously never overlap with UU or UA genotypes. The distribution of DN indicating distinct genotype groups is in close agreement to the established methods that were tested (Fig. 2) and compared favorably with published methods (3, 6, 8, 9).

Analysis time is <6 min and the DN method does not require specialized knowledge of the Dimension instrument. The simple procedure of replacing a tablet with a solution of 2 mmol/L dibucaine in well 6 of the CHE Flex is the only modification necessary for the user to perform; otherwise, the DN method is practically as easy to perform as the other methods on the Dimension. In addition, the DN method has a very small sample volume requirement (2 μL of sample plus 30 μL of dead volume) compared with the double 100-μL, 200-μL, 20-μL, and 200-μL volumes, respectively, required for measuring inhibited and uninhibited CHE rates with the two sample methods (6–9). In the

Fig. 2. Comparison of DN results between methods by simple linear regression for 53 clinical samples: (A) Dimension-ES vs reference method (7); (B) ace method (8) vs reference method (7); and (C) Dimension-ES vs ace method (8).
discrimination between genotype groups, the advantage of using the assessment of DN over CHE activity alone is clearly demonstrated by comparing the SDs between genotype groups in Table 1. The DN method performed equally well at a different site with a Dimension-AR instrument.

By virtue of its speed, efficiency, simplicity, and reliability, we conclude that this new DN method is appropriate for clinical use.

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References