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Quality assurance in age estimation based on aspartic acid racemisation

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Abstract Estimates of the age of living and dead individuals, obtained in order to answer legal or social questions, require minimum quality standards in order to guarantee data quality. We present an outline strategy (with recommendations) for the attainment of quality assurance in age estimation based on aspartic acid racemisation. The strategy is based on a definition of minimum standards for laboratories, including documentation of procedures, methodology and levels of expertise, and the formulation of guidelines for intralaboratory and interlaboratory quality control.

Key words Age estimation · Aspartic acid racemisation · Minimal standards · Quality control

Introduction

Age estimation may play a central role in the clarification of questions which have a major legal and/or social impact for the individual, as well as for the community. In such a framework, methods for age estimation have to fulfil specific demands which have been detailed recently (Ritz-Timme et al. 2000). Specifically, the methods must have been presented to the scientific community, as a rule by publication in peer-reviewed journals, clear information concerning the accuracy of age estimation by the method should be available, the methods need to be sufficiently accurate and in cases of age estimation in living individuals, principles of medical ethics and legal regulations have to be adhered to. However, even the application of methods that fulfil these demands cannot guarantee optimal results, if essential standards of procedures are not defined and if quality control is inadequate. At a time in which quality assurance has achieved great importance in all fields of the biomedical sciences, few attempts have been made to find common standardisation, calibration and evaluation procedures for methods for age estimation. With good reason, Wu et al. (1999) stated that:

"expert witnesses have an ethical responsibility that should not be taken lightly."

and that

"witnesses should ensure that their opinions are congruent with current scientific standards."

At present, there are no generally accepted guidelines concerning quality assurance in age estimation. Therefore efforts in this direction are necessary in order to guarantee quality standards for the important legal and social issue of age estimation in forensic medicine.

The aim of this article is to propose guidelines for quality assurance in age estimation based on aspartic racemisation in dentine. The proposed strategy of quality assurance considers the recommendations already published for forensic toxicology (Aderjan et al. 1998; Christophersen and Morland 1994; Wu et al. 1999) and is based on the definition of minimum standards and the formulation of demands on quality control.

Minimum standards

The defined (obligatory) standards are summarised and commented in the following.

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General demands and prerequisites

The personnel involved have to be trained and have access to an appropriate and properly maintained laboratory and equipment.

The method has to be correctly established taking into account those factors which may influence the outcome of the analysis (described in detail by Waite et al. 1999). A basic calibration has to be performed to check the quality of the method under the specific conditions of the laboratory. The verification of the correct establishment of the method by basic calibration is obligatory since the method comprises methodological details that influence the outcome of the analysis (summarised by Waite et al. 1999). Basic calibration will involve analysis of a sufficiently large number of teeth from individuals of known age followed by an adequate statistical evaluation of the relationship between the extent of aspartic acid racemisation and age. The resulting basic calibration data should ideally be available for each tooth type (Waite et al. 1999).

All details of the procedure, all data and the results of age estimation must be provable. The entire procedure has to be standardised by the introduction of common operation procedures. All methodological details should be properly documented in a laboratory manual and the analyst has to keep a record of all relevant data (e.g. names of the responsible personnel, the time course of the analysis, special features, all values including all calibration data).

Quality control has to be performed using adequate methods.

Methodology

A review of the methodological aspects of aspartic acid racemisation analysis for use in forensic science (Waite et al. 1999) has already identified methodological details which may influence the outcome of the analysis, namely details of the sampling strategy, the washing procedure, the pulverisation of dentine, the preparation of protein fractions, the hydrolysis and derivatisation conditions and the chromatographic analysis. Based upon this theoretical background (Waite et al. 1999), minimum standards for all relevant methodological steps and also for the interpretation of results are defined in this article. It is not the intention of this paper to recommend a single particular method but to formulate standards that should be considered if age estimation based on aspartic acid racemisation in dentine is used in a forensic framework.

- Choice of samples (total dentine, protein fractions after extraction)

Total dentine and an acid-soluble (“non-collagenous”) protein fraction can be analysed but total dentine is the most robust sample. The acid-soluble (“non-collagenous”) fraction yields a faster rate of racemisation. However, in cases with long post-mortem intervals selective leaching of this relatively small protein pool may change its overall character and thereby alter the extent of racemisation (Waite et al. 1999). For this reason the acid-soluble (“non-collagenous”) fraction should be analysed only in combination with the total dentine sample. The acid-insoluble (“collagenous”) fraction is not recommended for age estimation because the racemisation rates are very low in this protein fraction due to the preponderance of triple helical collagen (Collins et al. 1999).

- Sampling strategy

Teeth should be stored dry at a low temperature (–20 °C). Storage in fixative (in particular formaldehyde) should be avoided (Waite et al. 1999). All dental findings should be documented in detail. If the tooth morphology is of interest for later or other investigations, a radiograph should be taken and a tooth model should be produced. The types of teeth analysed must be identified and documented. The preparation of dentine samples with dental instruments has to be standardised, the influence of heat should be avoided. The dentine region analysed (e.g. root dentine, crown dentine, longitudinal sections) has to be clearly defined and prepared according to an exactly defined procedure.

- Washing procedure

All details of the washing procedure have to be defined exactly (e.g. solutions, volumes, washing times, etc.). Washing should be performed at 4 °C. Samples should not be washed with (even weak) acids to avoid a pre-extraction of acid soluble proteins.

- Pulverisation of dentine

Pulverisation of dentine for demineralisation and extraction of a “non-collagenous” protein fraction has to be highly standardised. Excessive grinding (which causes collagen chain scission) and the development of high temperatures during the pulverisation procedure (which promotes racemisation) should be avoided.

- Preparation of a “non-collagenous” protein fraction

For demineralisation and extraction of a “non-collagenous” protein fraction the HCl method is to be preferred to the EDTA method (Waite et al. 1999). A “non-collagenous” protein fraction should only be analysed if the conditions of extraction are exactly defined and standardised. Extraction should be performed at 4 °C.

- Hydrolysis, derivatisation and chromatographic analysis

These methodological stages have to follow an established standard protocol. The hydrolysis and derivatisation conditions must be optimised for the chromatographic procedure employed. The quality of derivatisation and chromatographic analysis has to be controlled by analysis of adequate standards in each series (see "intralaboratory quality control"). Measurements have to be performed at least in duplicate.

- Result interpretation (estimation of age from the measured *D*-aspartic acid/*L*-aspartic acid ratios)

The interpretation of results should be based on basic calibration data for each laboratory (see section "General demands and prerequisites"). Data from the literature can only be used for result interpretation if the basic calibration has proven their applicability. The estimation of age and the calculation of confidence intervals can be performed by means of inverse prediction (Zar 1984).

The expert opinion

The basic principles of the method should be explained in a understandable way, relevant literature should be cited. Methodological details have to be clearly and adequately presented, sufficient for assessment by other experts.

The presentation of the results has to include clear information about the "error" of the method, for example by giving the 95% confidence intervals associated with the predicted age (Zar 1984).

If any doubts about the reliability of the results arise (e.g. in cases with unknown post-mortem interval and environmental conditions) they should be highlighted and the implications discussed.

Quality control

Guidelines for quality control are summarised and commented on in the following. Intralaboratory quality control is obligatory; interlaboratory quality control remains to be organised.

Intralaboratory quality control

The laboratory needs to demonstrate with each analysis batch that the quality of detection and quantitative evaluation is sufficient to guarantee reproducible and accurate results.

The following standards should be available for usage as calibrators and controls:

A. Defined mixtures of *D*- and *L*-aspartic acid

The amino acid mixtures are stable if stored freeze-dried at –20 °C (Table 1).

Table 1 *D-/L*-aspartic acid ratios of *D-/L*-aspartic acid standards for intralaboratory (and interlaboratory) quality control after different storage times: The amino acid standards are stable for at least 8 months; the coefficients of variation for the presented data for the different standards are 3.99%, 0.84%, 1.43%, 0.52%, 0.79% and 0.54% respectively. The *D-/L*-aspartic acid standards were produced as follows: standards with different defined *D-/L*-ratios (0.0111, 0.0532, 0.1115, 0.1817, 0.2510 and 0.3373) were prepared. Aliquots were stored freeze-dried at –20 °C for the given storage time

Storage period (months)	*D-/L*-aspartic acid ratios of *D-/L*-aspartic acid standards after different storage times					
	Standard 0.0111	Standard 0.0532	Standard 0.1115	Standard 0.1817	Standard 0.2510	Standard 0.3373
0	0.0115	0.0532	0.1114	0.1823	0.2510	0.3383
1	0.0110	0.0529	0.1137	0.1805	0.2492	0.3352
2	0.0110	0.0535	0.1117	0.1809	0.2520	0.3394
3	0.0102	0.0523	0.1098	0.1800	0.2535	0.3390
4	0.0108	0.0525	0.1140	0.1803	0.2543	0.3405
8	0.0112	0.0530	0.1112	0.1820	0.2499	0.3375

Table 2 *D-/L*-aspartic acid ratios in aliquots of a dentine pool standard for intralaboratory (and interlaboratory) quality control: Constant *D-/L*-aspartic acid ratios in all aliquots; the coefficient of variation for the presented data is 0.88%. The dentine pool was produced as follows: dentine of teeth with a dentine age of 40 years was washed and prepared according to an established standard procedure of Ritz et al. 1993. In a first analysis the *D-/L*-aspartic acid ratio in the total dentine of each tooth was determined separately. Only the dentine of teeth with values within one standard deviation around the mean value was included in the dentine pool and pulverised. The dentine powder of all included teeth was combined, mixed and stored at –20 °C in aliquots of 10 mg as "dentine pool standard" samples

Aliquot	*D-/L*-aspartic acid ratios
1	0.0507
2	0.0502
3	0.0506
4	0.0500
5	0.0494
6	0.0505
7	0.0504
8	0.0501
9	0.0499
10	0.0509

B. Dentine samples from dentine pools with known *D-/L*-aspartic acid values

Pulverised dentine from teeth with identical dentine age can be combined to dentine pools with known *D-/L*-aspartic acid values; analysis of aliquots of such pools reveals constant values (Table 2). Dentine pools can be produced as described in the legend of Table 2.

C. Teeth from individuals of known age

Intralaboratory quality control should be performed according to a documented protocol and should consist of the following:

– In each set of samples, standards of types A-C (see above) must be included as controls to test the quality of all methodological steps. The analysis of standards of type A tests the quality of the chromatographic procedure, standards of type B serve to control the quality (especially the reproducibility) of the preparation of protein fractions, the hydrolysis and the derivatisation, and standards of type C are necessary to control the standardisation of tooth preparation, washing and pulverisation of dentine.

– The precision has to be controlled in the series and between series by repeated analysis of standards of type A (chromatographic precision) and type B (precision of sample processing). If possible, interlaboratory comparisons should be performed routinely (to test comparative precision). The coefficient of variation can be used as a measure of the precision; the maximal tolerable imprecision has to be defined.

– Standards of type C from individuals with different ages covering the whole age range have to be analysed in each series for final calibration. This tests if the basic calibration data (see above: "General demands and prerequisites") can be transferred to the actual series. Maximum tolerable discrepancies between the basic calibration data and the final calibration data have to be defined.

Interlaboratory quality control

External quality control could be achieved by the performance of interlaboratory exchanges (blind trials) and by the establishment of reference laboratories. Until now, these important tools of quality control have not been organised and efforts in these directions are necessary.

Final remarks

This proposed strategy for quality assurance in age estimation based on aspartic acid racemisation has been presented intentionally in a very general manner. The concrete realisation depends on the individual organisation of each laboratory and is the duty of the responsible scientist/expert.

References

Aderjan R, Briellmann T, Daldrup T, Demme U, Harzer K, Herbold M, Käferstein H, Kauert G, von Meyer L, Möller M, Mußhoff F, Schmitt G, Weinmann M (1998) Richtlinien der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen. Toxichem Krimtech 65: 18–24

Christophersen AS, Morland J (1994) Drug analysis for control purposes in forensic toxicology, workplace testing, sports medicine and related areas. Pharmacol Toxicol 74: 202–210

Collins MJ, Waite ER, van Duin ACT (1999) Predicting protein decomposition, the case of aspartic acid racemization kinetics. Phil Trans R Soc Lond B Biol Sci 354: 51–64

Ritz S, Schütz HW, Peper C (1993) Postmortem estimation of age at death based on aspartic acid racemization in dentin: its applicability for root dentin. Int J Legal Med 105: 289–293

Ritz-Timme S, Cattaneo C, Collins M, Waite ER, Schütz HW, Kaatsch H-J, Borrman HIM (2000) Age estimation: The state of the art in relation to the specific demands of forensic practise. Int J Legal Med 113: 129–136

Waite ER, Collins MJ, Ritz-Timme S, Schütz HW, Cattaneo C, Borrman H (1999) A review of the methodological aspects of aspartic acid racemization analysis for use in forensic science. J Forensic Sci 103: 113–124

Wu AHB, Hill DW, Crouch D, Hodnett CN, McCurdy HH (1999) Minimal standards for the performance and interpretation of toxicology tests in legal proceedings. J Forensic Sci 44: 516–522

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice Hall, Englewood Cliffs, pp 276–277