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Sampling and storage conditions influencing the measurement of parathyroid hormone in blood samples: a systematic review

Abstract: Parathyroid hormone (PTH) is relatively unstable: optimisation of pre-analytical conditions, including specimen type, sampling time and storage conditions, is essential. We have undertaken a systematic review of these pre-analytical conditions. An electronic search of the PubMed, Embase, Cochrane, Centre for Research and Dissemination and Bandolier databases was undertaken. Of 5511 papers identified, 96 underwent full text review, of which 83 were finally included. At room temperature PTH was stable in ethylenediaminetetraacetic acid (EDTA) preserved whole blood for at least 24 h and in EDTA plasma for at least 48 h after venepuncture. Losses were observed in clotted blood samples after 3 h and in serum after 2 h. At 4°C PTH was more stable in EDTA plasma (at least 72 h) than serum (at least 24 h). Central venous PTH concentrations were higher than peripheral venous concentrations. In the northern hemisphere, PTH concentrations were higher in winter than summer. PTH has a circadian rhythm characterised by a nocturnal acrophase and mid-morning nadir. Data related to frozen storage of PTH (–20°C and –80°C) were limited and contradictory. We recommend that blood samples for PTH measurement should be taken into tubes containing EDTA, ideally between 10:00 and 16:00, and plasma separated within 24 h of venepuncture. Plasma samples should be stored at 4°C and analysed within 72 h of venepuncture. Particular regard must be paid to the venepuncture site when interpreting PTH concentration. Further research is required to clarify the suitability of freezing samples prior to PTH measurement.

Keywords: chronic kidney disease; parathyroid hormone; plasma; rhythm; serum; temperature; whole blood.

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Introduction

Parathyroid hormone (PTH) is an 84 amino acid peptide hormone which has important physiological roles in regulating bone metabolism. It stimulates renal reabsorption of calcium, bone resorption and activation of vitamin D, while also inhibiting renal phosphate reabsorption, bone formation and bone mineralisation. PTH measurement is integral to the diagnosis and management of hypoparathyroidism and hyperparathyroidism. Patients with chronic kidney disease (CKD), which is associated with progressive loss of renal mass and consequent reduction in the activation of vitamin D [1], may develop chronic kidney disease-mineral bone disorder (CKD-MBD). Current guidelines recommend that PTH should be maintained within two to nine times the upper limit of the reference interval in CKD-MBD patients [2].

Optimal implementation of such guidelines requires not only that PTH methods give comparable results, which at present they do not [3, 4], but also that pre-analytical conditions are clearly defined. Results from proficiency testing schemes demonstrate marked between-method differences in PTH results which parallel the more than three-times the differences that can be observed in the same sample from patients with CKD-MBD [5]. Such variability represents significant clinical risk, which could result in opposite decisions, e.g., concerning whether or not a patient receives vitamin D treatment, cinacalcet or parathyroidectomy, depending upon the PTH method used.

Almost all clinical laboratories now use second or third generation PTH methods (Table 1). Factors likely to contribute to observed between-method variation include differences in method calibration, analytical specificity and assay design. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has established a Working Group [4], whose remit is to encourage manufacturers to calibrate their PTH assays in terms of the newly established recombinant International Standard for PTH (IS 95/646) (provided its commutability can

Table 1 Characteristics of PTH immunoassay methods.

	Availability	Antibody specificity	Cross-reactivity	Comment
1st generation [Radioimmunoassay]	1960s to 1980s	Polyclonal; directed against mid- or C-terminal region	C-terminal metabolic degradation fragments	No longer used
2nd generation [Two-site immunoassay]	1987 – ongoing	Two monoclonal antibodies directed against N- and C-terminal regions	Large C-terminal fragments including PTH 7-84	Commonly erroneously referred to as "intact PTH" assays. Fragments detected have a longer $t_{1/2}$ than intact PTH and are present in high concentration in blood of patients with kidney failure
3rd generation [Two-site immunoassay]	1999 – ongoing	Two monoclonal antibodies directed against first 6 N-terminal residues and against C-terminal region (39-84)	Thought to be more specific for intact PTH	Not yet widely used in clinical laboratories

PTH, parathyroid hormone.

be confirmed), to develop a reference measurement procedure for PTH, and to prepare a panel of reference plasma samples. This should ultimately improve between-method comparability of PTH methods.

As an important first step, and under the auspices of the IFCC PTH Working Group, the systematic review of published data reported here has enabled identification of optimal pre-analytical sampling and storage conditions for PTH, with the aim of producing good practice guidance for PTH measurement. The systematic review addresses the following three questions, developed according to the well-established population, intervention, comparator, outcome (PICO) approach [6]:

1. How stable is PTH in ethylenediaminetetraacetic acid (EDTA) or lithium heparin anticoagulated human whole blood or plasma, as compared to clotted whole blood or separated serum at room temperature, 4°C, −20°C and −80°C?
2. Does the site of sampling affect measured PTH concentration?
3. Does the time of sampling affect measured PTH concentration?

Results of the systematic review are reported here together with some suggestions for additional issues requiring further investigation.

Materials and methods

Electronic searches of the Medline, Embase, Cochrane, Bandolier and Centre for Research and Dissemination databases were undertaken to identify relevant articles (up to 06 December 2012). Medical subject headings (MeSH) terms and free text as well as the full search strategy used are presented in Supplemental Data Table 1. Searches were further expanded by the inclusion of relevant papers that were either known to the authors, had been referenced in the original articles or had been retrieved from related articles. Only full papers and letters were included in the search. Titles and abstracts were read and relevant papers obtained and reviewed by two authors (EH, EJL). A manual search of abstracts from national meetings of the Association for Clinical Biochemistry (Focus 1987–2012) and the American Association of Clinical Chemistry (AACC, 1993–2012) was also performed, with abstracts including PTH, parathyroid hormone, parathormone or parathyrin in the title being selected for review. We then undertook forward searching of abstracts to identify whether these had subsequently been published as full papers.

The following inclusion criteria were used: human studies addressing the stability of PTH in blood samples ex vivo, the effect of the sampling site on PTH concentration in blood samples ex vivo, and the effect of seasonal, circadian and/or ultradian (cycles shorter than 24 h) rhythms on PTH concentration in blood samples ex vivo. Exclusion criteria included research in animals, PTH (1–34), parathyroid hormone-related peptide (PTHrP), PTH receptor, PTHrP gene, PTH mRNA, PTH-receptor antagonist and preproPTH. For the first of the PICO questions addressed, publications prior to 1987 were excluded as they referred to earlier less specific PTH assays [7, 8].

The authors agreed and trialled spreadsheet fields. Data were extracted from the selected papers by EH and

checked by EJL. In some cases, confirmation of data was sought from authors through direct contact (e.g., e-mail). Data extracted included the number, nature and concentration range of samples, sample types studied and tube manufacturer, sample processing and storage conditions, assay method and manufacturer, comparator/reference used in each study, statistical analysis and a summary of findings including any information relating to circadian rhythm, pulsatile frequency and peak amplitude. The principal outcome measure was change in PTH concentration expressed in pmol/L or occasionally as a percentage. (N.B. 1 pmol/L is approx. equivalent to 9.43 ng/L).

Currently recommended methodological systems of assigning level of evidence and strength of recommendation could not easily be adopted to assess the evidence we identified [9]. Such systems have primarily been designed to assess evidence from therapeutic trials [10] or diagnostic studies [11] as opposed to pre-analytical sampling practices. Furthermore, for most of the data we identified it was not possible to produce quantitative summaries across studies. We have used a transparent evidence-based approach to review systematically, appraise critically and then discuss the evidence, including highlighting the strengths and limitations of the evidence and identifying gaps in the knowledge base behind the recommendations. In assessing the literature, for each of the three PICO questions we considered study design, internal validity and consistency across studies and directness.

Most studies were prospective with clearly reported protocols, so the risk of bias was considered low. The risk of allocation bias was also low, particularly in studies addressing PICO question 1 where samples from all subjects within individual studies were compared under the same conditions (e.g., storage temperature, sample type and tube type). Strongest weight was placed on those studies which we considered to equate with "level 1" evidence as described by Hayes et al. [11] (evidence from a single, high-powered, prospective, controlled study that is specifically designed to test a marker) or with "high level" evidence as described in the GRADE approach [10]. For PICO questions 2 and 3 all studies were observational cohort studies. When grading our recommendations we chose to use the approach described by Kidney Disease: Improving Global Outcomes (KDIGO) guideline group which acknowledges some of the restrictions of other systems when applied to data such as ours [12]. Recommendations were classified as either "strong", indicating a choice that most well-informed people would make, or "weak", indicating a choice that a majority of well-informed people would make but a substantial minority would not: recommendations were consequently worded as "We recommend…" or "We suggest…" respectively [12].

With respect to sample stability, the time points presented in the text and figures are the longest times for which PTH was reported to be stable in each study. Conclusions with respect to stability are conservative estimates taking all relevant studies into account. In addition to the literature search, information was acquired from manufacturers product inserts for several commonly used commercial PTH immunoassays.

The systematic review was written in accordance with preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [13] and a completed PRISMA checklist is presented in Supplemental Data Table 2.

Results and discussion

Search results

The electronic search identified 5478 papers (Figure 1). Thirty-three additional articles referenced in these papers were also included. Thirty-three meeting abstracts were also identified as relevant to the study of which five had subsequently been published. Of these five, four had already been identified by the search. One potentially relevant paper was excluded as it contained insufficient detail for data assimilation. A total of 96 papers were selected for full text review, of which 83 were finally included. Most
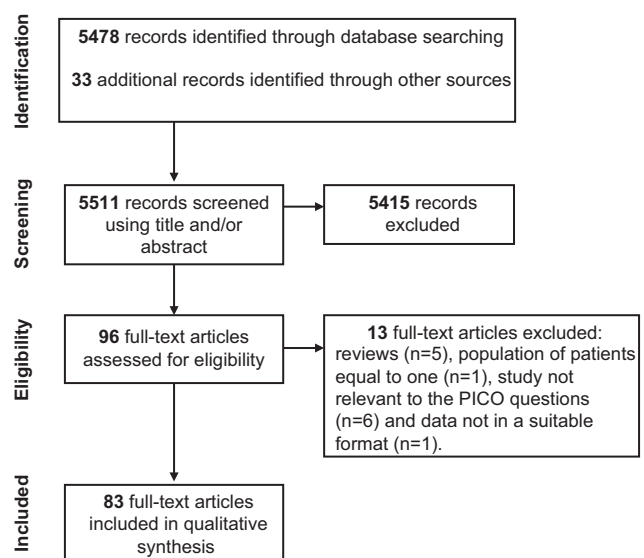
Figure 1 Flow diagram of the study selection process.

studies identified by the search were performed using second generation assays. As described in the Methods section, for the first PICO question the search was limited to papers published after 1987 as data obtained using earlier assays [7, 8] were considered to be of limited relevance to contemporary clinical practice.

Variability in study design

Although PTH stability ex vivo has been extensively studied, authors have not always reached the same conclusions. Assimilating and collating data from different studies was complicated by the different approaches taken. Confounding factors included variability in the length of time and specific time points over which stability was studied, variability in the time delay between venepuncture and separation of serum/plasma from cellular components of blood, missing data for PTH stability at specific time points and for specific types of samples, and/or failure in some studies to extend the observation period for sufficient time to enable identification of differences between sample types and/or comparison with baseline results. The practice recommendations, described below and summarised in Table 2, focused on results from studies in which there were direct comparisons between sample types, and those studies that answered questions most relevant to clinical practice.

Stability of PTH in whole blood, serum and plasma

PICO Question 1: *In human blood samples, how stable is PTH in EDTA or lithium heparin whole blood or plasma compared to clotted whole blood or separated serum at room temperature, 4°C, −20°C and −80°C?*

Studies focusing on PTH stability in blood samples were performed with different types of blood collection tubes: plain tubes, tubes containing anticoagulant (potassium-EDTA, citrate or lithium heparin) and/or gel separating tubes (GST) containing a barrier gel to separate serum from cellular blood constituents (cells and protein clot) after centrifugation. One study used results from plasma or serum from fresh whole blood as comparator [14], but most used results from serum or plasma which had been separated and frozen prior to analysis. For the following analysis, data have been grouped according to sample type and assay characteristics (i.e., second or third generation).

Stability in anticoagulated and clotted whole blood at room temperature

Fifteen papers relating to PTH stability in whole blood were identified that had used a second generation assay (Figure 2, Supplemental Data Table 3) [15–29]. Collation of study results was difficult due to variation in experimental design (see above), but almost all studies suggested that PTH was more stable in whole blood samples containing EDTA than in anticoagulant-free samples (Supplemental Data Table 3). More definitive evidence to support this was provided by seven studies that assessed the stability of different sample types within the same study using second generation methods [15, 16, 18, 22, 25, 26, 29] and one study using a third generation method [30] (Table 3). These data suggested that PTH was stable in EDTA whole blood for at least 24 h at room temperature as compared to only 3 h in clotted whole blood.

Table 2 Good practice recommendations for blood collection for PTH measurement developed as a result of the present systematic review.

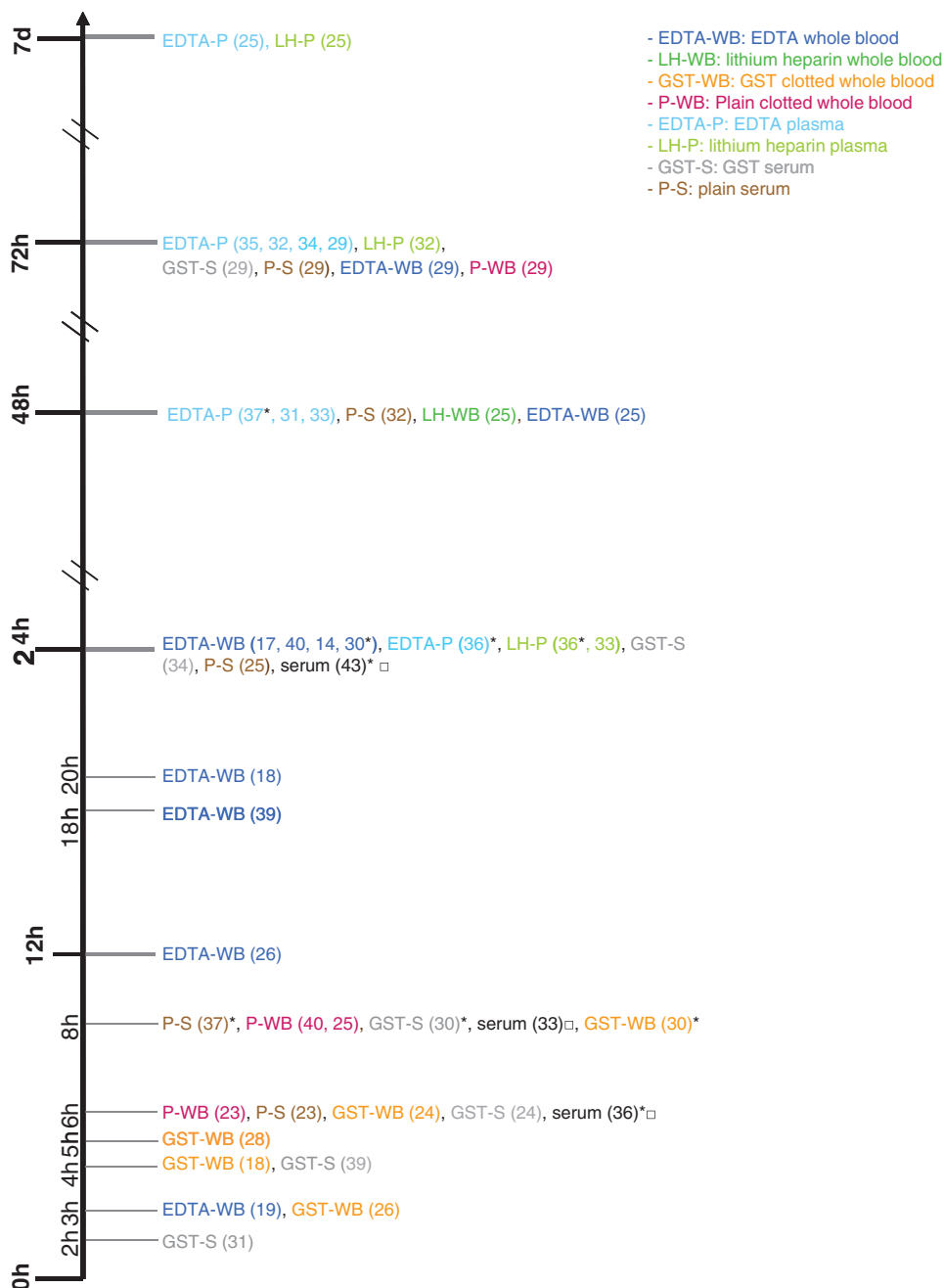|	
Recommendation 1: Type of sample tube	We recommend blood samples for PTH measurement should be taken into tubes containing EDTA and the plasma separated from the cells within 24 h of venepuncture [Strong recommendation].
Recommendation 2: Sample storage	We recommend EDTA plasma samples for PTH measurement should be stored at 4°C and analysed within 72 h of venepuncture [Strong recommendation].
Recommendation 3: Site of sampling	We recommend blood samples for PTH measurement should always be collected from the same sample site (central or peripheral) for comparison both within and between individuals. Clinical guidelines should explicitly state whether targets refer to peripheral or central venous concentrations [Strong recommendation].
Recommendation 4: Seasonal variation	We suggest season, latitude and vitamin D status should be considered and/or reported in all studies undertaking reference range determinations for PTH and when interpreting PTH results in individual patients [Weak recommendation].
Recommendation 5: Time of collection	We suggest blood samples for PTH measurement should be collected between 10.00 and 16.00 and results interpreted against a reference interval derived for this sampling time [Weak recommendation].

EDTA, ethylenediaminetetraacetic acid; PTH, parathyroid hormone.

Table 3 Data supporting the recommendations' regarding storage conditions and sample collection.

Storage temperature	Sample type	Stability (h) in tubes with anticoagulant as indicated		Patient group (number)	Method (generation)	Year [Reference]
		None	EDTA			
RT	Blood	3	12	Kidney failure receiving haemodialysis (8)	Siemens Immulite (2nd)	2000 [26]
RT	Blood	4	20	Kidney failure receiving haemodialysis (17)	Siemens ADVIA Centaur (2nd)	2007 [18]
RT	Blood	<8	24	Kidney failure receiving haemodialysis (16)	Roche Elecsys (2nd)	2007 [16]
RT	Blood	8	24	Kidney failure receiving haemodialysis (11)	Siemens Immulite (2nd)	2003 [15]
RT	Blood	8	24	Kidney failure receiving haemodialysis (15)	DiaSorin Liaison (1-84) (3rd)	2012 [30]
RT	Blood	8	48ᵃ	Metabolic bone disease (18)	Roche Elecsys (2nd)	2011 [25]
RT	Blood	<18	18	Kidney failure receiving haemodialysis (31)	Roche Elecsys (2nd)	2009 [22]
RT	Blood	6	72	Healthy individuals	Roche Cobas (2nd)	2012 [29]
RT	Separated serum or plasma	2	48	Kidney failure receiving haemodialysis (13)	Nichols ICMA (2nd)	2003 [31]
RT	Separated serum or plasma	<6	72ᵃ	Hyperparathyroidism (9); healthy individuals (8); acute pancreatitis (3)	Nichols IRMA (2nd)	1994 [32]
RT	Separated serum or plasma	8	48ᵃ	Healthy individuals and chronic kidney disease patients (15)	Roche Elecsys (2nd)	2002 [33]
RT	Separated serum or plasma	24	72	Chronic kidney disease (31)	Siemens Immulite 2000 (2nd)	2005 [34]
RT	Separated serum or plasma	24	168ᵃ	Metabolic bone disease (18)	Roche Elecsys (2nd)	2011 [25]
RT	Separated serum or plasma	<72	72	Not specified (36)	Siemens Immulite (2nd)	2002 [35]
RT	Separated serum or plasma	6	24ᵃ	Healthy individual (2) and hyperparathyroid patients (1)	In-house IRMA (3rd)	2001 [36]
RT	Separated serum or plasma	8	48ᵃ	Kidney failure receiving haemodialysis (17)	Nichols Bio-Intact PTH (3rd)	2004 [37]
RT	Separated serum or plasma	8	24	Kidney failure receiving haemodialysis (15)	DiaSorin Liaison (1-84) (3rd)	2012 [30]
RT	Separated serum or plasma	6	72	Healthy individuals	Roche Cobas (2nd)	2012 [29]
4°C	Blood	72	72	Healthy individuals	Roche Cobas (2nd)	2012 [29]
4°C	Separated serum or plasma	72	72	Healthy individuals	Roche Cobas (2nd)	2012 [29]
4°C	Separated serum or plasma	24ᵇ	72	Healthy individuals and chronic kidney disease patients (15)	Roche Elecsys (2nd)	2002 [33]
4°C	Separated serum or plasma	55	120ᵃ	Not specified (minimum of 6)	Nichols IRMA (2nd)	2001 [38]
4°C	Separated serum or plasma	72	72	Chronic kidney disease (31)	Siemens Immulite 2000 (2nd)	2005 [34]
4°C	Separated serum or plasma	168	672ᵃ	Metabolic bone disease	Roche Elecsys (2nd)	2011 [25]
4°C	Separated serum or plasma	24	24ᵃ	Healthy individual (2) and hyperparathyroid patients (1)	In-house IRMA (3rd)	2001 [36]
4°C	Separated serum or plasma	48	48	Kidney failure receiving haemodialysis (15)	DiaSorin Liaison (1-84) (3rd)	2012 [30]

ᵃSimilar stability was observed for EDTA and heparin plasma samples; ᵇsimilar stability was observed for serum and heparin plasma samples. EDTA, ethylenediaminetetraacetic acid; GFR, glomerular filtration rate.

Legend:
- EDTA-WB: EDTA whole blood
- LH-WB: lithium heparin whole blood
- GST-WB: GST clotted whole blood
- P-WB: Plain clotted whole blood
- EDTA-P: EDTA plasma
- LH-P: lithium heparin plasma
- GST-S: GST serum
- P-S: plain serum

Timeline points (room temperature, latest point with no significant difference):

- 7d: EDTA-P (25), LH-P (25)
- 72h: EDTA-P (35, 32, 34, 29), LH-P (32), GST-S (29), P-S (29), EDTA-WB (29), P-WB (29)
- 48h: EDTA-P (37*, 31, 33), P-S (32), LH-WB (25), EDTA-WB (25)
- 24h: EDTA-WB (17, 40, 14, 30*), EDTA-P (36)*, LH-P (36*, 33), GST-S (34), P-S (25), serum (43)* □
- 20h: EDTA-WB (18)
- 18h: EDTA-WB (39)
- 12h: EDTA-WB (26)
- 8h: P-S (37)*, P-WB (40, 25), GST-S (30)*, serum (33)□, GST-WB (30)*
- 6h: P-WB (23), P-S (23), GST-WB (24), GST-S (24), serum (36)*□
- 5h: GST-WB (28)
- 4h: GST-WB (18), GST-S (39)
- 3h: EDTA-WB (19), GST-WB (26)
- 2h: GST-S (31)
- 0h

Figure 2 PTH stability at room temperature in whole blood, serum and plasma (latest point at which no significant difference to baseline was observed).
References are cited in parentheses; *, 3rd generation assay; □, exact clotted sample type (i.e., plain or GST tube) not specified by authors. GST, gel separating tubes.

## Stability in separated serum and plasma at room temperature

Eighteen papers studying PTH stability in separated serum and plasma using second generation (Supplemental Data Table 4) [19, 20, 22–25, 27, 29, 31–35, 38–42] and four using third generation (Supplemental Data Table 5) [30, 36, 37, 43] assays were identified. PTH stability in EDTA plasma and serum was directly compared in seven studies using second generation assays [25, 29, 31–35]. In these, PTH was more stable in EDTA plasma than in serum (Table 3). In one study slight increases in measured PTH concentration in

EDTA plasma after 24 and 48 h were observed, although it was concluded overall that PTH was stable for 72 h in EDTA plasma but for only 24 h in serum [34]. Overall, these results suggested that PTH was stable in separated EDTA plasma for at least 48 h at room temperature but that in serum significant immunoreactivity may be lost after as little as 2 h.

Stability at other temperatures

Only one study directly compared the stability of PTH in clotted and EDTA whole blood at 4°C: PTH was reported stable for 72 h at 4°C in both sample types [29] (Figure 3). PTH was also reported to be stable in EDTA whole blood
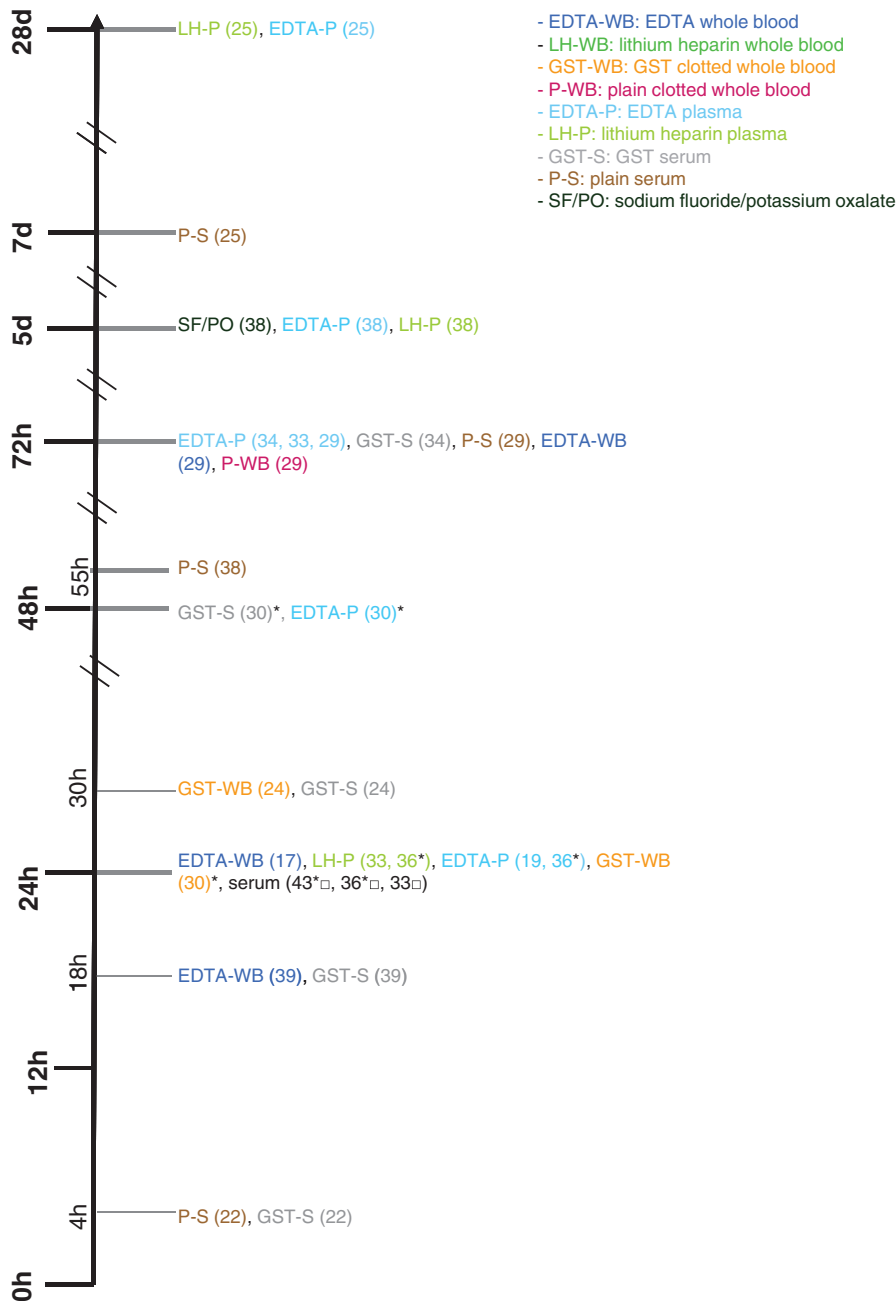
Figure 3 PTH stability at 4°C in whole blood, serum and plasma (latest point at which no significant difference to baseline was observed). References are cited in parentheses; *, 3rd generation assay; □, exact clotted sample type (i.e., plain or GST tube) not specified by authors; GST, gel separating tubes.

for at least 18 h [22] or 24 h [17] at 4°C and in clotted whole blood for 30 h [24] (Table 3, Supplemental Data Table 3). Nine studies addressed stability at 4°C in serum or plasma (Supplemental Data Table 4) [19, 22, 24, 25, 29, 33, 34, 38, 39]. Five of these directly compared the stability of PTH in serum and plasma at 4°C [25, 29, 33, 34, 38] using second generation assays generally confirming superior stability in EDTA (Table 3). In both sample types, PTH was more stable at 4°C than at room temperature.

Three studies addressed long-term frozen storage of PTH using second generation assays (Supplemental Data Table 4). When serum and EDTA plasma samples were kept at –20°C for 24 h, no difference in measured PTH was observed for the two sample types, while PTH degradation was significantly greater in EDTA plasma after 5 days at –20°C [16]. At –80°C, Brinc et al. [44] demonstrated instability of PTH after 2 months (up to 16% loss compared to baseline) in serum and EDTA plasma. Using third generation methods, PTH was reportedly stable for 14 days in serum [43] and for 12 months in serum and plasma kept at –20°C and –80°C [30]. Observed stability may be method dependent: Cavalier et al. [14, 16] reported that if stored at –20°C or –80°C PTH was more stable in serum than EDTA plasma when measured using the DiaSorin Liaison method (9 vs. 2 months, respectively). In contrast, PTH was stable in both frozen serum and plasma for at least 2 years when analysed using the Roche Elecsys method. It is possible that this might reflect conformational alterations during freezing but further confirmatory studies are required.

Using a variety of assays, PTH withstood four (serum and EDTA plasma [36]) to six (serum [23, 43]) freeze-thaw cycles without measured recovery being affected.

Recommendations for sample handling

With respect to analyte stability ex vivo, most studies, with both second and third generation assays, indicate PTH to be more stable in EDTA whole blood than clotted whole blood [15, 16, 18, 25, 26, 29, 30], and in EDTA and lithium heparin plasma than in serum at room temperature [25, 31–35]. Whilst direct comparisons of lithium heparin whole blood with EDTA whole blood [25], and lithium heparin plasma with EDTA plasma [25, 32], suggested similar stability could be achieved in lithium heparin preserved tubes, there was limited evidence upon which to base a recommendation. Furthermore, one manufacturer (Diasorin) does not support the use of lithium heparin. The following statement therefore seems appropriate:

1. We recommend blood samples for PTH measurement should be taken into tubes containing EDTA and the plasma separated from the cells within 24 h of venepuncture [Strong recommendation].

This recommendation is consistent with guidance issued by the Clinical Laboratory Standards Institute [45] and the World Health Organization [46]. It is also broadly in accord with advice provided in manufacturers' kit inserts, most of which suggest measurement of PTH in either EDTA plasma or serum, but confirm that PTH is more stable in EDTA plasma than serum (Table 4, [35, 37, 47]). We accept that PTH is commonly measured in conjunction with calcium, and sometimes vitamin D, to permit interpretation and that this recommendation will necessitate an additional sample being taken since calcium cannot be measured in EDTA plasma. This is clearly a practical limitation, but it is not unique to assessment of bone mineral metabolism (e.g., assessment of the pituitary-adrenal axis requires different samples for ACTH and cortisol). Clearly under optimal sample handling conditions (<3 h between venepuncture and separation/measurement) clotted blood may also be a suitable sample [26], but such stringent requirements are not likely to be achievable in many clinical laboratories.

Once separated, PTH was more stable in EDTA plasma than in serum, but the stability of PTH in both sample types could be successfully extended by refrigeration.

While losses of PTH observed in clotted blood samples may be small within the time frame of a typical working day (e.g., 8% [16] or 10% [18] after 8 h; 10% after 12 h [26]), such differences could contribute to misdiagnosis or changes in management of patients. Given the many other factors that influence clinical interpretation of PTH results (e.g., between-method differences, specificity) [48], laboratory professionals should ensure samples are appropriately stored. Based on the evidence reviewed here, the following recommendation seems appropriate:

2. We recommend EDTA plasma samples for PTH measurement should be stored at 4°C and analysed within 72 h of venepuncture [Strong recommendation].

Historically many laboratories have measured PTH in batch mode following freezing of samples at –20°C for variable periods of time. Many manufacturers' recommendations will support this practice (Table 4). However, published evidence was inconsistent regarding the stability of PTH under frozen storage conditions. Should laboratories still need to freeze plasma prior to PTH measurement, we suggest that they establish the stability of PTH in frozen plasma as measured with their own assay.

Table 4 Summary of manufacturer's recommendations relating to PTH preferred sample types and their processing within the laboratory.

Manufacturer (analyser)	Method name (Product reference/code)	Assay generation	Sample type	Sample stability and storage	Long term storage	References
Abbott Diagnostics (Architect)	Intact PTH (8K25)	2nd	– Serum (use of GST may result in a decrease in concentration) – Plasma samples (lithium heparin, sodium heparin or potassium EDTA tubes) – Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens – Do not use sodium citrate, sodium fluoride/potassium oxalate, and ammonium heparin tubes	– PTH stable on or off the clot, or red blood cells for up to 2 days at 4°C – If testing delayed by more than 2 days, remove serum or plasma from the clot or red blood cells and store frozen	At least 6 months	Not mentioned
Roche Diagnostics GmbH (modular)	Intact PTH	2nd	– Use preferably EDTA plasma, as PTH is more stable in plasma than serum – If serum is used, centrifuge the blood immediately	– PTH stable for 8 h at RT, 2 days at 4°C (serum) – PTH stable for 2 days at RT, 3 days at 4°C (plasma)	6 months at −20°C (serum and plasma)	Not mentioned
Siemens Healthcare Diagnostics Ltd (Advia Centaur)	Intact PTH (iPTH, 06587575)	2nd	– EDTA plasma or serum recommended – For patient sample comparison, use the same specimen type throughout	– EDTA plasma stable for 8 h at RT, 72 h at 4°C – Serum for 4 h at RT, 48 h at 4°C	– PTH stable at −70°C for 8 months (EDTA plasma) – Serum not tested	[35, 37, 47]
Beckman Coulter Inc (Access)	Intact PTH (A16972)	2nd	– Serum and plasma (heparin and EDTA) recommended	– PTH stable at RT for 8 h. Above 8 h, refrigerate samples at 4°C for no longer than 48 h (EDTA or heparin plasma) – PTH stable at RT for 4 h. For longer delays, refrigerate samples at 4°C for no longer than 8 h (serum)	Stable at −20°C or colder for no longer than 6 months (EDTA or heparin plasma, serum)	[47]
DiaSorin Inc (Liaison)	1–84 PTH Assay (310630)	3rd	– Serum or EDTA-plasma can be used – Serum to be separated from the clot as soon as possible – Do not use lithium heparin plasma – In a controlled study of 44 paired serum/EDTA-plasma samples, the serum samples returned higher values than EDTA-plasma by an average of 7.1% – Fasting samples recommended, but not required	– PTH stable at RT for 48 h (EDTA plasma), 72 h (EDTA whole blood) and 2 h (serum); – PT stable at 4°C for 72 h (EDTA plasma), 72 h (EDTA whole blood) and 72 h (serum)	−20°C or below	Not mentioned

(Table 4 Continued)

Manufacturer (analyser)	Method name (Product reference/code)	Assay generation	Sample type	Sample stability and storage	Long term storage	References
IDS PLC (IDS-iSYS)	PTH (1–34)	3rd	– Serum, plasma EDTA	– Samples should be separated as soon as possible after collection	–20°C or below	Not mentioned
Siemens Healthcare Diagnostics Ltd (Immulite 2000)	Intact PTH (L2KPP6)	2nd	– EDTA plasma or serum (with or without gel barrier) – Separate plasma and serum from the cells as soon as possible – EDTA plasma samples must be kept cold throughout the collection and separation process – Use a refrigerated centrifuge – EDTA collection tubes must be filled to their capacity. Failure to completely fill the tube will result in excess concentration of EDTA which will interfere with the assay, causing a false depression of values.	– PTH stable at 4°C for 72 h (EDTA plasma), for 48 h (serum)	Up to 2 months at –20°C	Personal Communication (Siemens)
Scantibodies Laboratory, Inc	Whole PTH (1–84) Specific ImmunoChemiluminoMetric Assay (ICMA, 3KG002)	3rd	EDTA-plasma	– PTH stable in whole blood at 4°C for up to 48 h – Plasma should be stored at -20°C or lower	Plasma should be stored at –20°C or lower	Not mentioned
Scantibodies Laboratory, Inc.	Whole PTH (1–84) Specific Immunoradiometric (IRMA) Bead Assay 3KG056	3rd	EDTA-plasma	– PTH stable in whole blood at 4°C for up to 48 h – Plasma should be stored at –20°C or lower	Plasma should be stored at –20°C or lower.	Not mentioned
Scantibodies Laboratory, Inc	Whole PTH (1-84) Specific IRMA (Coated Tube) 3KG014	3rd	EDTA-plasma or serum	– PTH stable in whole blood at 4°C for up to 48 h – Plasma should be stored at –20°C or lower	Plasma should be stored at –20°C or lower.	Not mentioned
Scantibodies Laboratory, Inc	Total Intact PTH IRMA (Coated Bead) 3KG600	2nd	EDTA-plasma	– PTH stable in whole blood at 4°C for up to 48 h – Plasma should be stored at –20°C or lower	Plasma should be stored at –20°C or lower.	Not mentioned
Scantibodies Laboratory, Inc	Total Intact PTH Assay IRMA (Coated Tube) 3KG013	2nd	EDTA-plasma or serum	– Plasma should be stored at –20°C or lower	Plasma should be stored at –20°C or lower.	Not mentioned

CKD, chronic kidney disease; EDTA, ethylenediaminetetraacetic acid; ICMA, immunochemiluminometric assay; IRMA, immunoradiometric assay; PTH, parathyroid hormone; RT, room temperature.

Influence of sampling site

PICO Question 2: *In human blood samples, does the sampling site affect PTH concentration?*

In clinical practice, most samples are taken from the antecubital vein. However, in haemodialysis patients, samples are often taken through a central line. In haemodialysis patients, PTH concentrations were found to be 30% higher in central blood (superior vena cava, median 24.3 pmol/L, interquartile range 9.2–38.2 pmol/L) compared to peripheral blood (forearm vein, median 15.3 pmol/L, interquartile range 6.3–29.0 pmol/L) [49] (Supplemental Data Table 6). Similarly in patients with primary hyperparathyroidism undergoing parathyroidectomy with intra-operative PTH monitoring, central venous (internal jugular vein) PTH concentrations were higher compared to peripheral venous PTH concentration (17.5 vs. 10.8 pmol/L [50]; 1.3–20.0 pmol/L higher [51]).

In relation to the above two clinical situations, particular regard must be paid to the site of origin of the blood samples. Use of central venous catheters in haemodialysis is variable but significant. For example, in the UK 65% of haemodialysis patients commenced dialysis in 2005 using a central venous catheter although by 12 months this had fallen to 30% [52]. In Canada between 2001 and 2004 the prevalence of catheter use in the haemodialysis population was 52% [53].

3. We recommend blood samples for PTH measurement should always be collected from the same sample site (central or peripheral) for comparison both within and between individuals. Clinical guidelines should explicitly state whether targets refer to peripheral or central venous concentrations [Strong recommendation].

Influence of time of sampling

PICO Question 3*: In human blood samples, does the time of sampling affect PTH concentration?*

Seasonal variation

PTH concentrations in blood have been reported to fluctuate according to the season [54–57] (Supplemental Data Table 7). With the exception of one small early study using a first generation assay [58], all studies identified reported a relative decrease in PTH concentration in summer and an increase in winter [54–57]. Studies undertaken with second generation assays suggested the difference between mean winter and summer concentrations was <1 pmol/L [57, 59]. All studies were undertaken in the northern hemisphere.

It seems likely that the observed pattern may mirror and reflect seasonal variation of vitamin D concentration [59, 60]. Given the clear inverse relationship between PTH and vitamin D it has been suggested that reference ranges for PTH should be established in vitamin D-replete individuals [60, 61]. However, this remains a controversial area. Whether the observed seasonal variation in vitamin D concentration is pathological and not normal physiology is difficult to assess. The definition of vitamin D sufficiency, often regarded as the concentration above which PTH cannot be suppressed further, varies widely (e.g., from 30 to 110 nmol/L [60]) and indeed, in the largest study to date, no threshold above which increasing vitamin D concentration failed to further suppress PTH could be identified [62]. Furthermore, the relationship between PTH and vitamin D was highly dependent on age [62]. The population in which PTH is most commonly measured is also known to have a high prevalence of vitamin D deficiency/insufficiency [63] and therefore use of a PTH reference range derived in vitamin D repleted individuals may be inappropriate.

4. We suggest season, latitude and vitamin D status should be considered and/or reported in all studies undertaking reference range determinations for PTH and when interpreting PTH results in individual patients [Weak recommendation].

Circadian variation

Studies addressing circadian variation of PTH are summarised in Supplemental Data Table 8 [64–90]. Most studies reported a circadian bimodal rhythm with a nocturnal acrophase, a mid-morning nadir and a smaller afternoon peak (Table 5). Peak times varied between studies and were affected by gender [75, 77]. There was also interindividual variability in the return to baseline (between 06:00 and 10:00) [81, 91]. Most studies reported a circadian amplitude amongst healthy individuals of between 0.3 and 0.8 pmol/L [64, 66, 68, 69, 72, 73, 75, 77, 79, 81, 82, 90] although higher amplitudes of 1.2 [73] and 1.9 [68] pmol/L, respectively, were also reported. Circadian variation was absent in patients with thalassemia [70] and primary hyperparathyroidism [80, 81].

Logue et al. [81] recommend that blood samples should be collected between 10:00 and 16:00 and results interpreted against a reference range based on this

Table 5 Summary of included studies on circadian variation of PTH.

Authors	Night Peak	Day Peak	Acrophase	Nadir
Ahmad et al. [64]	22.30–06.00	14.00–22.00	02.00	08.00–10.00
Arnaud et al. [65]	12.00–20.00	—	20.00	08.00
Calvo et al. [67]	02.00–04.00	18.00–20.00	18.00 and 02.00	10.00 and 20.00
Chapotot et al. [68]	23.00–07.00	15.00–23.00	—	—
El-Hajj et al. [69]	01.00–03.00	17.30	03.14	10.00–11.00
Fraser et al. [71]	22.00–07.00	14.00–22.00	06.12	09.30–10.30
Generali et al. [72]	—	—	17.15	—
Greenspan et al. [74]	03.20	15.20	—	12.00
Greenspan et al. [75]	23.35 ± 05.25 ♀, 20.30 ± 02.10 ♂	—	—	—
Herfarth et al. [76]	No night samples	P.M.	14.00	09.30
Joseph et al. [77]	22.00–24.00 ♀, 24.00–02.00 ♂	14.00–17.00 ♀♂	09.46–13.15 ♀, 10.45–13.25 ♂	—
Jubiz et al. [78]	02.00–04.00	—	—	08.00
Kitamura et al. [79]	A.M.	—	23.00 ± 4	—
Logue et al. [82]	02.00–06.00	—	04.05	10.00
Logue et al. [81]	A.M.	—	04.05	09.30
Markowitz et al. [83]	04.00	20.00	—	—
Nielsen et al. [84]	P.M.	—	16.30	10.30
Nielsen et al. [85]	23.30–07.30	—	—	09.30
Rejnmark et al. [87]	—	—	20.00	10.00
White et al. [90]	24.00–04.00	14.00–18.30	01.00–02.00	08.00

EDTA, ethylenediaminetetraacetic acid; GST, gel separating tubes; ICMA, immunochemiluminometric assay; PTH, parathyroid hormone; RT, room temperature.

sampling time. Since the data were derived using first or second generation assays, it is possible that the reported diurnal variation of PTH could reflect differential clearance of PTH fragments over a 24 h period, e.g., with reduced renal clearance of (7-84) PTH at night reflecting decreased glomerular filtration rate at night [92].

5. We suggest blood samples for PTH measurement should be collected between 10:00 and 16:00 and results interpreted against a reference interval derived for this sampling time [Weak recommendation].

We found no studies that addressed the relative diagnostic accuracy of PTH measurement at different time of the day. Generally studies were undertaken in small cohorts and no studies specifically addressing this question in CKD patients were identified. Partly because of this there are concerns about the validity of the data identified and we have only made a weak recommendation. Our recommendation is based upon practical considerations since it avoids the times of day over which PTH peaks are observed.

Pulsatile variation

Studies addressing pulsatile variation of PTH are summarised in Supplemental Data Table 9 [68, 76, 79, 93–102].

As for many peptide hormones, a pulsatile secretory pattern was superimposed on the circadian rhythm of PTH. Thirteen studies addressed this issue. In all but one [76], PTH secretion was found to be pulsatile with one to seven secretory pulses per hour [68, 79, 94–96, 98, 99, 101, 102]. Reported pulse amplitudes were 0.5 [68], 0.8 [79] and 1.8 [96] pmol/L.

The ability to detect secretory pulses depends on the frequency of blood sampling, which varied greatly between studies. Not all studies covered an entire 24 h period and no studies were undertaken using a third generation assay. Most data suggested that pulse amplitude was similar in magnitude to the effects of circadian variation noted above, approximately 1 pmol/L. In clinical practice it is not possible to mitigate against the effects of pulse amplitude in terms of measuring PTH and this probably contributes to the high biological variation observed for PTH [103, 104]. The effects of pulsatile secretion should be considered when interpreting PTH results (e.g., by recommending confirmatory sampling when appropriate). In the future this may suggest a role for other bone markers (e.g., bone alkaline phosphatase), which might provide a more time-averaged measure of PTH effect, analogous to the use of insulin-like growth factor 1 (IGF1) measurement to integrate the pulsatility of growth hormone secretion [105].

Other pre-analytical factors influencing PTH measurement

We have focused on pre-analytical sampling and storage conditions for PTH. However, a number of studies describing other pre-analytical influences on PTH concentration were identified. Food ingestion may [106] or may not [79] affect PTH concentration. In many of the studies reported above [64, 66, 67, 69, 71, 74, 75, 77, 79, 83–85, 87, 89, 90] related to circadian variation, subjects were provided with meals at specified times of day: no clear relationship between feeding and PTH concentration was reported. Fasts of 33 h and 96 h led to the loss of PTH circadian rhythm [107, 108]. A vegetarian diet led to a higher PTH concentration compared to a meat diet (5.9 vs. 4.9 pmol/L [109]). The reported effect of strenuous exercise was inconsistent [110–113], and sleep did not seem to affect the circadian and/or pulsatile release of PTH [68]. Higher mean PTH concentrations were reported in men compared to women (4.4 vs. 2.8 pmol/L) [75], in African-American women compared to white women (2.0 vs. 3.3 pmol/L) [66] and in postmenopausal females compared to males and premenopausal females (5.8 pmol/L vs. 5.4 and 4.7 pmol/L, respectively) [77].

Limitations

There were many limitations to the studies we included. Most used as comparator a sample which had been frozen at baseline, as opposed to a freshly analysed sample, used by one study only [14], so interpretation of test results could have been confounded by changes in PTH concentration following freezing. Different approaches were taken to define significance of change, e.g., some studies used a prespecified percentage change compared to baseline [16, 17, 25, 29, 33, 38, 44], whilst others used paired statistical analyses [15, 18, 19, 21–24, 26, 28, 30–32, 34, 35, 37, 39, 40, 42, 43]. PTH as measured by most clinical assays is not a single entity with assays recognising different molecular forms to different extents and the prevalence of these forms varying between individuals and disease states. Most studies were performed on samples from patients with kidney disease or hyperparathyroidism, and we cannot be confident therefore that our conclusions are necessarily generalisable to other patient groups or healthy individuals. Nevertheless in the majority of studies, PTH was more stable in EDTA whole blood or plasma than clotted whole blood or serum, irrespective of experimental design.

We found no direct published comparisons of clotted and EDTA whole blood stability at 4°C. Data with respect to stability of serum and plasma at –20°C and –80°C were extremely limited and we are unable to draw conclusions in this regard. We were somewhat surprised by this given the relative consistency of manufacturer's recommendations in this respect (Table 4) and the fact that, until relatively recently, many laboratories analysed PTH in batch mode with frozen storage prior to analysis. We may have missed such evaluation data by limiting our searches to the era of second and third generation PTH assays. However, we consider that data derived using earlier PTH assays is not reliable in this respect. Clinical laboratories and researchers undertaking studies where samples are to be stored frozen should establish analyte stability for their own sample types and assays.

Second and third generation PTH assays measure different mixtures of peptides which may have differing stability [114, 115]. We found no direct published comparisons of observed analyte stability with second versus third generation PTH assays but there were some data to suggest that PTH may be more stable when measured by third rather than second generation assays [37]. This could be explained on the basis that the peptide fragments detected by the second generation assays may be less stable than the intact molecule detected by third generation assays. If confirmed, this should provide further impetus to support the use of third generation assays.

Conclusions and suggestions for further study

Pre-analytical sampling and storage conditions affecting PTH concentration have been systematically reviewed and recommendations for good practice developed (Table 2). Several areas of uncertainty remain which should be the subject of further research. These include: 1) how stability of PTH in EDTA whole blood as measured with a third generation assay compares with that as measured with a second generation assay; 2) the circadian and seasonal variation of PTH as measured with a third generation assay; 3) how CKD and vitamin D status affect these rhythms; and 4) further data are also required regarding the stability of PTH at –20°C, –30°C (i.e., below the eutectic point) and –80°C with both second and third generation

assays. There is also an urgent requirement for sound reference range data derived under clearly defined sampling conditions. For the present useful progress can be made by adopting and implementing the recommendations in Table 2. This should significantly improve the comparability and consistency of PTH data in all clinical settings.

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