

# Neonatal blood gas sampling methods

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Blood gas sampling is part of everyday practice in the care of babies admitted to the neonatal intensive care unit, particularly for those receiving respiratory support. There is little published guidance that systematically evaluates the different methods of neonatal blood gas sampling, where each method has its individual benefits and risks. This review critically surveys the available evidence to generate a comparison between arterial and capillary blood gas sampling, focusing on their relative accuracy and complications, as well as briefly mentioning the management of such complications. This evidence-based summary and guidance should help inform best practice in the neonatal intensive care unit, and minimise the exposure of babies to unnecessary and potentially serious risk.

The most accurate and non-invasive method of measuring oxygenation is oxygen saturation monitoring. Indwelling arterial catheters are a practical, reliable and accurate method of measuring acid-base parameters, provided they are inserted and maintained with the proper care. Capillary blood gas sampling is accurate, and a good substitute for radial 'stab' arterial puncture, avoiding many of the complications of repeated arterial puncture.

*You work in a regional neonatal intensive care unit. An 8-day-old premature baby with a right radial arterial catheter develops severe ischaemia of the right hand.*

*The baby was born at 28 weeks' gestation with a birth weight of 1 100 g. He had been ventilated since the first few hours of life, initially for severe hyaline membrane disease subsequently complicated by nosocomial pneumonia. A radial arterial catheter was sited on day 6, before which multiple repeated radial 'stab' arterial blood gases had been performed.*

*You immediately remove the radial arterial catheter and begin vasodilatory treatments including warming of the contralateral upper limb, application of 2% nitroglycerin ointment and axillary brachial plexus sympathetic nerve block. However, the ischaemic injury to the right hand does not resolve, and after a further 10 days necessitates amputation of the 3rd, 4th and 5th digits.*

*You wonder whether anything could have been done to prevent this baby's peripheral vascular injury, and what the evidence base is with regard to the different methods of neonatal blood gas sampling.*

Blood gas sampling and acid-base determination are critical tools in the assessment and monitoring of neonates with respiratory or circulatory compromise, in particular those receiving respiratory support. Blood gas measurement is also part of the basic assessment of most metabolic conditions, including renal, liver and endocrine abnormalities as well as inborn errors of metabolism.

There are various methods for obtaining a blood gas sample from a neonate. This article aims to survey the evidence regarding the accuracy, use and complications of the commonly used methods of neonatal blood gas sampling in order to make best practice recommendations. Brief guidance is also given on the management of complications arising from blood gas sampling. While non-invasive techniques such as transcutaneous blood gas monitoring are discussed in part, the focus is on systematic review of the evidence concerning the relative benefits and harms of invasive methods of blood gas sampling.

## Search strategy

### Primary sources

Ovid MEDLINE (1948 - 2011) was searched using the following search terms: (blood gas OR blood sampling) AND (capillary OR arterial OR indwelling OR umbilical OR puncture) including related terms. The search was limited to human biology, newborn population and English language. This yielded 252 results, from which 17 studies were selected. Similar additional searches were performed during the review as the relevance of additional topics such as pulse oximetry and transcutaneous blood gas measurement emerged. Further studies were found on examination of the reference lists of the included papers.

### Secondary sources

The Cochrane database yielded 3 reviews using the same search criteria as above, and an additional review was found later during a search for reviews concerning neonatal procedural pain.

## Which methods of neonatal blood gas measurement can be used?

Methods of neonatal blood gas measurement include:<sup>1</sup>

- indwelling arterial catheters, e.g. umbilical or peripheral arterial line (which also allow invasive blood pressure monitoring)
- peripheral arterial 'stab' puncture sample
- capillary blood sample (commonly taken from a heelstick)
- non-invasive methods: oxygen saturation monitoring, end-tidal carbon dioxide (CO<sub>2</sub>) monitoring and transcutaneous oxygen tension/CO<sub>2</sub> monitoring.

## Which method gives the most accurate information?

Acid-base information obtained from blood gas samples taken from an indwelling arterial catheter appears to be the gold standard,<sup>1</sup> and is the method by which alternative methods (i.e. capillary blood gas sampling) are compared in the literature. Standard information included in most invasive blood gas measurements consists of pH, partial pressure of oxygen (pO<sub>2</sub>), partial pressure of carbon dioxide (pCO<sub>2</sub>), bicarbonate (HCO<sub>3</sub>) and base excess.

**Oxygenation (O)**

Invasive blood gas sampling is not the best way to assess oxygenation in a neonate. There is a weak correlation of pO<sub>2</sub> between capillary samples and arterial blood taken from indwelling arterial lines.<sup>2-4</sup> However, even pO<sub>2</sub> measurement in blood samples taken from indwelling arterial lines only yields single-point pO<sub>2</sub> measurement, and does not offer dynamic and continuous information on oxygenation. Oxygenation should be monitored by non-invasive measurement of oxygen saturation (SpO<sub>2</sub>), which is continuous, reliable, and should be available as the standard of care in all institutions caring for sick newborns.<sup>5</sup> Carefully titrated oxygen therapy with the aim of targeting a pre-defined range of oxygen saturations may be important in preventing free radical damage from oxygen toxicity such as retinopathy of prematurity.<sup>6</sup> Transcutaneous oxygen tension monitoring (TcPO<sub>2</sub>) has been suggested as an alternative to SpO<sub>2</sub> monitoring, but there is insufficient evidence to prove that it is any better in terms of reducing morbidity.<sup>7</sup> Other problems associated with TcPO<sub>2</sub> monitoring include skin burns, as well as the need for frequent calibration and rotation of monitoring sites.<sup>8</sup>

**Ventilation and acid-base (pH, CO<sub>2</sub>, HCO<sub>3</sub>)**

A meta-analysis has helped establish the strong correlation of pH, pCO<sub>2</sub> and HCO<sub>3</sub> between arterial and capillary blood in adults.<sup>9</sup> Courtney *et al.* have systematically reviewed the neonatal literature published before 1990 concerning the correlation between arterial and capillary blood gas measurements, as well as performing their own study.<sup>10</sup> Almost all the studies demonstrated strong correlation between arterial and capillary pH, and HCO<sub>3</sub> if measured. Employing a tabular format, the authors describe results of 10<sup>2,3,11-18</sup> of the 14 relevant studies as demonstrating ‘good’,

‘close’ or ‘satisfactory’ correlation between arterial and capillary measurements of pCO<sub>2</sub>.

There is variation in the study populations and methodology across the studies included in the review by Courtney *et al.*,<sup>10</sup> making comparisons difficult. Most of these variations are discussed in the review. However, an additional observation is that only 4<sup>12-14,19</sup> of the studies contain data from preterm infants. Additionally, although most of the studies attempt to induce vasodilatation at the intended site of capillary puncture, their methods vary from different ways of warming the skin<sup>2,10-12,14,17,19,20</sup> to vasodilatory creams and iontophoretic techniques.<sup>3,15,16</sup> Capillary and arterial samples were not taken simultaneously in 5<sup>11-13,17,21</sup> out of the 14 studies, which as Courtney *et al.* note,<sup>10</sup> could potentially affect the comparison of arterial and capillary measurements. Potentially painful procedures such as capillary and arterial ‘stab’ puncture blood gas sampling have been shown to cause measurement bias by decreasing oxygenation/ventilation during the procedure itself secondary to crying.<sup>22</sup> However, overall it is difficult to quantify how such methodological differences might specifically contribute to differences between the results.

There is also variation in the methods used to express results across the studies, some<sup>2,11-14,20</sup> quoting the mean difference (with standard error) between paired capillary and arterial measurements for pH and pCO<sub>2</sub>, while other studies<sup>3,16,17</sup> calculate the correlation coefficient (*r*). Two of the studies do not employ any numerical or statistical analyses, exclusively presenting their data visually on scatter plots.<sup>15,19</sup> Courtney *et al.* identify 4 studies that do not recommend the use of capillary blood gas sampling in neonates on the basis of poor observed power

**TABLE 1. REVIEW OF NEONATAL AND PAEDIATRIC LITERATURE POST-1990 CONCERNING CORRELATION OF PAIRED CAPILLARY AND ARTERIAL MEASUREMENTS OF PH AND PCO<sub>2</sub>**

Citation	Study population	Methodology	Results (correlation between capillary and arterial measurements)	Statistical significance
Saili <i>et al.</i> , <sup>23</sup> 1992	51 neonates with moderate birth asphyxia of gestational age 32 - 38 weeks, and postnatal age of 48 - 72 hours	Simultaneous paired arterial and capillary samples Capillary samples obtained after foot warming	pH <i>r</i> =0.92 pCO <sub>2</sub> <i>r</i> = 0.32	<i>p</i> <0.05 for both pH and pCO <sub>2</sub>
Harrison <i>et al.</i> , <sup>24</sup> 1997	50 PICU patients aged from 1 month to 220 months with various pathologies, 53% of patients ventilated	Simultaneous paired arterial and capillary samples No extremity warming prior to capillary sampling	pH <i>r</i> <sup>2</sup> =0.903 pCO <sub>2</sub> <i>r</i> <sup>2</sup> =0.955	<i>p</i> <0.0001 for both pH and pCO <sub>2</sub>
Escalante-Kanashiro <i>et al.</i> , <sup>25</sup> 2000	75 samples from PICU patients from 0.6 - 134 months (including 8 neonates) with various pathologies	Simultaneous paired arterial and capillary samples Capillary samples obtained after finger warming	pH <i>r</i> =0.87 pCO <sub>2</sub> <i>r</i> =0.86 (correlation reduced in the presence of hypotension <i>r</i> =0.52, but not altered by poor perfusion or hypothermia/pyrexia)	No quoted measure of statistical significance
Yang <i>et al.</i> , <sup>4</sup> 2002	33 premature infants admitted to NICU, birth weight range 635 - 2 500 g	Capillary blood taken 5 minutes after arterial sampling No extremity warming prior to capillary sampling	pH <i>r</i> =0.92 pCO <sub>2</sub> <i>r</i> =0.93	No quoted measure of statistical significance
Yildizdaş <i>et al.</i> , <sup>26</sup> 2004	116 samples from PICU patients (including 8 neonates) with varying pathologies, 28% of patients ventilated	Simultaneous paired arterial and capillary samples Capillary blood taken from heel in infants and finger of children No extremity warming prior to capillary sampling	pH <i>r</i> =0.823 pCO <sub>2</sub> <i>r</i> =0.988 (correlation unchanged by poor perfusion, hypotension or hypothermia/pyrexia)	pH <i>p</i> <0.001 pCO <sub>2</sub> <i>p</i> <0.001

PICU = paediatric intensive care unit; NICU = neonatal intensive care unit.

**TABLE 2. REVIEW OF NEONATAL LITERATURE CONCERNING COMPLICATIONS OF DIFFERENT METHODS OF BLOOD GAS SAMPLING WITH SUGGESTED PREVENTION AND MANAGEMENT STRATEGIES**

Adverse event	Procedure	Evidence	Strategies to avoid or treat adverse event
Pain	Capillary heelstick Arterial puncture	Systematic review <sup>34</sup>	Breastfeeding or supplemental breastmilk <sup>35</sup> Non-nutritive sucking <sup>36</sup> Sucrose solution <sup>37</sup>
Bruising	Capillary heelstick	Neonatal case reports <sup>39,40</sup>	Use of an automated (spring-loaded) incision device reduces bruising compared with a conventional manual lancet <sup>41</sup>
Calcaneal osteomyelitis (can result in flatfoot and calcaneal deformity – case reports <sup>38</sup> )	Capillary heelstick	Neonatal case report <sup>44</sup>	Optimal depth of lancet puncture 0.85 mm <sup>42</sup> Site of lancet puncture <sup>43</sup> (see diagram in 'How to obtain a heelstick capillary sample')
Calci-fied cutaneous heel nodules	Arterial puncture	Neonatal case reports <sup>45</sup>	None found
Haematoma	Arterial puncture	Neonatal case reports <sup>47</sup>	Apply direct pressure to site after puncture Rotate puncture sites <sup>46</sup>
Pseudo-aneurysm	Arterial puncture	Neonatal case report <sup>47</sup>	Some suggestion that repeated puncture should be avoided <sup>47</sup>
Arteriovenous fistula formation	Radial arterial puncture	Neonatal case report <sup>48</sup>	Some suggestion that repeated puncture should be avoided <sup>48</sup>
Carpal tunnel syndrome	Radial arterial puncture	Neonatal case report <sup>45</sup>	None found
Median nerve damage	Brachial arterial puncture	Neonatal case reports <sup>49</sup>	Avoid brachial artery puncture due to high (13%) incidence of median nerve damage <sup>49</sup>
Infiltration and extravasation injury	Peripheral arterial catheter	Neonatal case reports <sup>50</sup>	Management depends on severity; options include: observation alone, impregnated occlusive dressings (e.g. hydrocolloids) or irrigation with 0.9% saline or hyaluronidase <sup>51</sup>
Haemorrhage	Peripheral arterial catheter Umbilical arterial catheter	Neonatal case reports <sup>52</sup>	Use of three-way tap systems for sampling <sup>52</sup>
Cumulative blood loss from repeated sampling	All sampling methods	Neonatal observational study <sup>53</sup>	Rational ordering of blood tests <sup>54</sup> Blood sample tubes with ideal fill lines <sup>54</sup>
Retrograde embolisation	Peripheral arterial catheter	Neonatal experimental study <sup>55</sup>	Use of small volume flushes (0.5 - 1 ml is suggested), <sup>56</sup> which are injected slowly
Cerebral embolisation	Temporal arterial catheter	Neonatal case reports <sup>57</sup>	Avoid temporal arterial catheterisation <sup>56</sup>
Arrest of growth plate	Peripheral arterial catheter	Neonatal case reports <sup>58</sup>	None found
Infection (superficial abscess)	Peripheral arterial catheter	Neonatal case reports <sup>52</sup>	Breadth of topic beyond scope of this review. In brief, helpful measures include: <sup>59</sup> strict hand hygiene for all involved in care, strict aseptic technique during insertion of line, minimise unnecessary access ports, regular change of ports/giving sets, sterilise ports before access, removal of line when not necessary
Infection (bacteraemia)	Peripheral arterial catheter Umbilical arterial catheter	Review article <sup>59</sup> (which quotes audits and retrospective studies)	Umbilical arterial catheter – continuous infusion of heparinised saline (0.25 - 1 U/ml) reduces thrombotic risk <sup>60</sup>
Catheter occlusion and thrombosis	Umbilical arterial catheter	Systematic review <sup>60</sup>	Peripheral arterial catheter – continuous infusion of heparinised saline (1 U/ml) reduces thrombotic risk <sup>61</sup>
For umbilical arterial catheter also includes:	Peripheral arterial catheter	Meta-analysis which includes neonatal studies <sup>61</sup>	For extensive or limb-threatening thrombosis consider thrombolysis with recombinant tissue-type plasminogen activator (rTPA) <sup>62</sup>
• aortic thrombosis			
• hypertension and haematuria			

**CONTINUED: TABLE 2. REVIEW OF NEONATAL LITERATURE CONCERNING COMPLICATIONS OF DIFFERENT METHODS OF BLOOD GAS SAMPLING WITH SUGGESTED PREVENTION AND MANAGEMENT STRATEGIES**

Adverse event	Procedure	Evidence	Strategies to avoid or treat adverse event
Vascular compromise: • arterial occlusion and transient ischaemia • severe ischaemia, extremity necrosis and gangrene For umbilical arterial catheter also includes: • gluteoperitoneal necrosis ± sciatic nerve palsy • spinal cord injury and flaccid paralysis • increased risk of necrotising enterocolitis	Peripheral arterial catheter  Umbilical arterial catheter	Literature review of neonatal observational studies <sup>63</sup> Neonatal case reports <sup>52,64,65</sup>  Systematic review of effects of umbilical catheter position <sup>67</sup> Neonatal case reports <sup>70,71</sup>	Regular monitoring of extremities for signs of vascular compromise <sup>66</sup> Prompt catheter removal when no longer necessary <sup>66</sup> Malposition is an important risk factor for umbilical arterial catheter ischaemic and thrombotic complications - high umbilical catheter position (T6 - 9) reduces the risk of ischaemic and thrombotic complications <sup>67</sup> Vascular spasm is common and usually resolves within minutes. <sup>66</sup> If persistent signs of vascular compromise detected, remove catheter immediately and consider: • contralateral limb warming <sup>62</sup> • 2% nitroglycerin application <sup>68</sup> • sympathetic nerve blocks (e.g. brachial plexus) <sup>69</sup> • surgical management as a last resort
Refractory hypoglycaemia	Umbilical arterial catheter	Neonatal case reports <sup>72</sup>	Avoid umbilical arterial position near vessels supplying pancreas (T11 - L1) and avoid glucose containing umbilical arterial perfusate <sup>72</sup>
Increased risk of intraventricular haemorrhage	Umbilical arterial catheter		Slow withdrawal of blood samples (over at least 40 seconds) <sup>73</sup>

PICU = paediatric intensive care unit; NICU = neonatal intensive care unit.

of capillary measurements for predicting arterial pCO<sub>2</sub>. At least 2<sup>10,20</sup> of these studies (including Courtney *et al.*'s own) do in fact demonstrate correlation between the arterial and capillary pCO<sub>2</sub> measurements, but they are of less clinical value owing to wide scatter limits.

Our literature search did not reveal any additional studies published before 1990 examining arterial and capillary correlation for pCO<sub>2</sub> and pH, except for the 14 studies in the comprehensive review by Courtney *et al.*<sup>10</sup> Since 1990 there have only been 2 comparable studies in the neonatal population, and 3 comparable studies in the paediatric population. Table 1 demonstrates that 4 of these 5 studies published after 1990 show strong correlation between paired capillary and arterial measurements of pCO<sub>2</sub>. All studies demonstrate good correlation between paired capillary and arterial measurements of pH, and HCO<sub>3</sub> if measured.

In summary, systematic review of all the neonatal literature concerning paired capillary and arterial measurements reveals unanimously good correlation for pH, and good correlation in most studies for pCO<sub>2</sub>. There are difficulties in speculating as to why 3 studies<sup>20,21,23</sup> failed to demonstrate good pCO<sub>2</sub> correlation, as these studies do not commonly share any variation in population characteristic or methodology different from the rest of the studies. Our review therefore concludes that capillary blood gas sampling can be used to measure pCO<sub>2</sub> accurately.

Non-invasive CO<sub>2</sub> monitoring consists of two techniques: end-tidal CO<sub>2</sub> monitoring (ETCO<sub>2</sub>) and transcutaneous CO<sub>2</sub> monitoring (TcPCO<sub>2</sub>). Molloy *et al.*<sup>27</sup> performed a review of neonatal non-invasive CO<sub>2</sub> monitoring, and highlighted that 2 out of the 3 studies they reviewed demonstrated good correlation between ETCO<sub>2</sub> and arterial pCO<sub>2</sub>. They comment, however, that ETCO<sub>2</sub> lacks precision, and therefore measurement may be more useful for screening purposes or trending. There are several studies investigating the accuracy of TcPCO<sub>2</sub>, and after due consideration Molloy *et al.*'s review concludes that TcCO<sub>2</sub> performs better than ETCO<sub>2</sub> with regard to correlation with arterial pCO<sub>2</sub>.<sup>27</sup> The general limitations of transcutaneous monitoring highlighted above such as skin burns apply to TcCO<sub>2</sub> monitoring.

**Other biochemical parameters and pre-analytical considerations**

Other biochemical parameters can be measured using capillary blood and show strong correlation with arterial blood, such as haematocrit, haemoglobin, sodium, calcium, glucose, bilirubin and lactate.<sup>4,28,29</sup> Haemolysis from the capillary sampling method is likely to be responsible for the poorer correlation between arterial and capillary blood for potassium and chloride.<sup>4</sup> Such haemolysis is more likely in the presence of polycythaemia,<sup>30</sup> and should prompt collection of a free-flowing venous or arterial sample. Polycythaemia is also associated with spurious hypoglycaemia, and anaemia can likewise give rise to falsely elevated glucose readings.<sup>31</sup>

The presence of hypothermia, hyperthermia or increased capillary refill time does not appear to affect the accuracy of capillary blood gas results.<sup>26</sup> Indeed, warming the heel before heelstick capillary sampling does not appear to increase accuracy.<sup>2,32</sup> The presence of hypotension may affect the accuracy of results, which should prompt consideration of arterial sampling.<sup>25</sup> Pre-analytical considerations are also important when sampling from indwelling arterial catheters. It has been suggested that for a neonatal indwelling arterial catheter with a dead-space volume of 0.6 ml, at least 1.6 ml of blood should be withdrawn before collection of a blood gas sample to avoid contamination errors from the flush/perfusate.<sup>33</sup>

**What are the complications associated with the various sampling methods?**

Invasive blood gas sampling is associated with a wide array of complications. The majority of the neonatal evidence comes from case reports; however, there are some observational studies and even systematic reviews, although

these tend to concern prevention or management of complications. The results of our literature search are presented in Table 2, which shows that capillary heelstick sampling is associated with fewer and less serious adverse effects than arterial sampling.

Repeated radial arterial 'stab' puncture has been described as 'difficult, dangerous and unpractical'.<sup>13</sup> Table 2 demonstrates there is some evidence for this claim, particularly since capillary sampling is associated with fewer adverse events which can be prevented more easily. In general, the complications associated with indwelling arterial catheters are serious in nature, and arterial catheters should therefore be removed without delay when no longer required.<sup>66</sup>

Indwelling arterial catheters can be inserted peripherally (radial, posterior tibial or dorsalis pedis) or centrally in the umbilical artery. Table 2 outlines the evidence behind the recommendation that catheterisation of the brachial and temporal arteries should be avoided. There is also opinion that ulnar artery catheterisation is similarly risky owing to the possibility of ulnar nerve damage or abnormal or compromised collateral blood supply of the hand.<sup>56,74</sup> It has also been suggested that use of the Allen test in detecting adequate collateral circulation before radial arterial puncture may not be a reliable predictor of subsequent risk of vascular injury.<sup>75</sup>

## Conclusion and recommendations

Indwelling arterial catheters remain a practical, reliable and accurate method of neonatal blood gas sampling, provided they are inserted and maintained with the proper care. Capillary blood gases are accurate and a good substitute for radial 'stab' arterial puncture for most babies, avoiding many of the complications of repeated arterial puncture.

Based on our review of the evidence, we propose the following simple guideline for blood gas sampling in neonates:

### Proposed neonatal blood gas sampling guideline

1. Reasonable attempts should be taken to site indwelling arterial catheters\* (radial, posterior tibial, dorsalis pedis or umbilical – procedure described elsewhere<sup>76</sup>) *only* if the need for regular blood gas analysis is anticipated.

2. When indwelling arterial catheters are not feasible or not indicated because of infrequent sampling, heelstick capillary blood gases should be the first-line sampling method for acid-base analysis.

3. Peripheral arterial 'stab' sampling has little place in neonatology.<sup>†</sup>

\*Caregivers must be informed of the benefits of catheterisation, as well as the common complications such as infection, haemorrhage and vascular injury. Catheters should be inserted in a safe and sterile manner, and removed as early as possible. Heparinised saline should be continuously infused, and no other fluid or medication should ever be given through the catheter. Nursing and medical staff must be vigilant in monitoring extremities to look for signs of vascular compromise.

†Arterial stabs should only be performed under the following circumstances:

- point measurement of pO<sub>2</sub> when oxygen saturation monitoring is unavailable or impossible
- acid-base information required in the clinical scenario of hypotension.

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## BEST PRACTICE

### How to obtain a heelstick capillary blood gas sample

(adapted from *Capillary Blood Sampling Guideline*, Great Ormond Street Hospital, London, 2010<sup>77</sup>)



1. Consider procedural analgesia before performing any painful procedure on a neonate. Options include breastfeeding, expressed breastmilk, or administration of 0.5 - 2 ml of 25% sucrose on the tongue 2 minutes before the procedure.<sup>78</sup>
2. Universal precautions should be observed during this procedure.
3. The baby's heel should be held with your non-dominant hand, with your fingers around the ankle and lower leg, while partly encircling the baby's heel with your thumb
4. Select an appropriate site for heelstick puncture:
  - a. the chosen site should not be extensively traumatised from previous heelstick puncture
  - b. vascular injury risk is reduced by puncturing the medial or lateral aspects of the heel
  - c. avoid the posterior and central regions of the heel, as puncture of these sites can cause damage to nerves, tendon, cartilage and bone
  - d. avoid inflamed/oedematous tissue.
5. Clean the site with an appropriate neonatal antiseptic solution (such as 0.5% chlorhexidine in 70% isopropyl alcohol<sup>79</sup>) and allow to dry.
6. Puncture the skin using an appropriate lancet device (depth 0.85 mm for a premature baby, 1.0 mm for a term baby).
7. Wipe away initial blood flow with cotton wool.
8. Maintain grip while gently compressing the heel to produce a droplet of blood.
9. Collect the droplet of blood using an appropriate capillary tube (pre-heparinised electrolyte-balanced heparin<sup>80</sup>).
10. Release compression while maintaining grip to allow reperfusion, and then re-compress to allow further formation of further droplets of blood.
11. Repeat until desired sample volume has been obtained.

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