Skin-Puncture and Blood-Collecting Technique for Infants: Update and Problems

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This is updated information on acceptable practice in skin puncture and blood collection in infants, as well as on the devices used, with the additional aim of emphasizing major problem areas and some tentative solutions. Consensus standards for skin puncture have little experimental support, and evade the hard fact that studies are needed to clarify optimum sites for puncture and depth and width of lancets, and to assess the effects of compression and skin resistance in the puncturing process. Preliminary data revealed that the puncturing depth of 2.4 mm recommended for the newborn is excessive. In four of 14 newborns at necropsy, the distance from posterior planar skin surface to underlying bone ranged between 2.0 and 2.2 mm. An experimental lancet, with a 1.8-mm tip length and a diameter of 0.79 mm yielded customary blood volumes from newborns in three of the four pediatric centers where it was tested. Lack of success with the lancet was attributed to inexperienced phlebotomists, not to the lancet's decreased size. Also reviewed are problems with common devices used, and the need for examining the "economy" of blood collection.

Additional Keyphrases: newborns · pediatric clinical chemistry · sample collection · sample handling

I wish to update the information in the second edition of Pediatric Clinical Chemistry (1), and offer some critical insight into current problems needing further exploration. Since 1981, the National Committee for Clinical Laboratory Standards (NCCLS), an American voluntary consensus group, has produced an Approved Standard (2). Similarly, a Scandinavian consensus group has published their recommendations for collection of blood by skin puncture, especially for providing reference values (3). The basis for much of today's acceptable practice in skin puncture arises from the unique anatomical studies of the newborn's heel reported by Blumenfeld et al. in 1979 (4).

Justification for skin puncture in pediatrics has been adequately outlined in the NCCLS publication, and will not be repeated here. One unstated point, however, is that accessible veins in the sick infant must be reserved exclusively for parenteral therapy, actual or potential. Almost all infants less than one month old admitted to pediatric intensive-care wards will receive such therapy, and certainly most sick infants older than two years will. Facts such as these arise from examining the "economy" of blood collection (5). Of 528 randomly selected requests for laboratory work in a pediatric hospital, 57.2% were for patients who were receiving intravenous fluids.

Economy in blood collection via skin puncture of the infant is required because:

- The volume of blood obtained per puncture is limited to about 400 μL.
- Minimizing the number of punctures is imperative. In 327 requests for laboratory tests on infants, 0–1 year old, the mean number of punctures was 1.4 (SD 0.7) or, in other words, for every 10 requests 14 punctures were required to provide the needed volume of blood. For older children (e.g., 2 patients, one to five years old), the mean number of punctures was 1.1 (SD 0.4), or 11 punctures per 10 requests.
- The mean number of laboratory tests per request was about three, depending on the system for counting tests (e.g., "blood gas" is either one or three tests: pH, pCO2, pO2).
- The frequency of repeat requests on a single patient is only vaguely known, as is also the total number of requests, punctures, and volume of blood drawn per hospital stay.

When repeatability of tests was defined as the percent probability that a test would be repeated on the same patient within a week, the repeatability was 79.3% (494 blood chemistry tests on 514 patients). A test was considered single if it was not repeated during the seven-day period.

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1 The information on equipment and devices mentioned in this article was obtained from responses to a detailed questionnaire sent to people at pediatric centers. The responders were: Gregory J. Buffone, Mary J. Hopkins, Clinical Chemistry, Texas Children's Hospital, Houston, TX; John P. Connelly, J. Earle, Cathy Warner, Dept. of Clin. Biochem., Royal Children's Hospital, Melbourne, Australia; Klaus Dörner, Klinikum der Christian-Albrechts Universität 20 Kiel Kinderklinik, Kiel, F.R.G.; Marshall F. Goren, A. Ossica, Dept. of Pathol. & Clin. Chem., St. Jude's Children's Research Hospital, Memphis, TN; Jocelyn M. Hicks, Dept. of Lab. Med., Children's Hospital National Med. Ctr., Washington, DC; Samuel Meites, Carllotta Thompson, Clin. Chem. Lab., Children's Hospital, Columbus, OH; John Sherwin, Timothy Zundell, Valley Children's Hospital, Fresno, CA; Charles P. Turley, Children's Hospital Laboratory, Little Rock, AR.

The mention of a commercial product in this article does not imply an endorsement by the American Association for Clinical Chemistry, or that equally useful products are unavailable on the market.

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covered. The repeatability of blood gas determinations was
>90%, of electrolytes, 80.5%, and of glucose, 87.1% (5).
Skin-puncture-derived blood is presumably subject to artefactual contamination from interstitial and intracellular fluids, and from hemolysis arising from ruptured erythrocytes, but scientific studies of these contaminants are woefully lacking. A recent examination of this problem indicated that after the first 13 postnatal days hemoglobin in skin-puncture-derived blood does not exceed 200 mg/dL, nor does the plasma K+ concentration increase, as would be expected from excessive extravasation of tissue fluid (6). During the first 13 postnatal days, when the erythrocyte is especially fragile, about 3% of 390 samples tested had hemoglobin concentrations exceeding 1000 mg/dL, the highest value being 1470 mg/dL. An inordinate amount of "squeezing" the heel was required to increase the K+ values by more than 0.2 mmol/L in two of six newborns. The tentative conclusion was that artefactual contamination with hemoglobin, K+, and lactate dehydrogenase in skin-puncture-derived blood from subjects older than 13 days was partly the result of the phlebotomist's inept technique. Poor handling of the specimen was also a factor. With proper technique, contamination by interstitial and intracellular fluids of samples obtained from younger patients is usually clinically negligible.

Methods for Skin Puncture and Blood Collection

General

Collecting trays. Various trays are used for conveying the paraphernalia of skin puncture. These may be hand-carried or placed on mobile carts. Phlebotomists use carts to accommodate a greater stock and assortment of supplies. Trays are usually constructed of metal or plastic but should be sterilizable. Among those reported are kitchen utility trays found in many variety stores (e.g., "Rubbermaid") and specifically designed products.

Prewarming the puncture site. For collecting samples to measure blood gases (pH, pCO2, pO2) in infants, prewarming the puncture site is essential. Warming increases the rate of arterial blood flow (reddening) in the area, thus helping ensure that the test data will compare validly with those for arterial blood; it also increases the volume of blood that can conveniently be collected. For tests other than blood gases, such warming makes blood collection easier (7). Several choices of warming materials are used. The most convenient is a cloth towel or washcloth soaked in running tap water at a temperature not exceeding 42 °C, preferably checked by a thermometer. Temperatures above 42 °C cause burns (3, 8). The patient's foot or hand is wrapped in the cloth for 3 to 5 min. The wrapped extremity may be encased in a plastic bag, to assure heat retention and to keep the patient's bed dry. The Scandinavian group cautions against using dry heat from lamps, or hyperemia-inducing ointments that may cause burns or are ineffective (3).

Site of puncture. The least-hazardous site for heel puncture of the neonate is medial to a line drawn posteriorly from the middle of the great toe to the heel, or lateral to a line drawn posteriorly from between toes four and five to the heel (2). In older infants, the palmar (fleshy) surface of the distal phalanx of the second, third, or fourth fingers may be punctured, the middle finger being the first choice. No puncture should ever be made in an edematous site, for obvious reasons; nor, to prevent risk of infection, should a previous puncture route be re-entered. The Scandinavian group has added an additional site for puncture, the medial part of the plantar arch of the foot in front of the calcaneal bone, but they do not mention any anatomical study in support of this choice (3). The NCCCLS report specifically proscribes use of the central area of the foot (area of the arch): "This may result in injury to nerves, tendons, and cartilage and offers no advantage over puncturing the heel."

Skin Puncture

Antiseptic. Isopropanol/water (70/30 by vol, "70%") remains the antiseptic favored for cleansing the puncture site. Several manufacturers sell sterile, disposable absorbent pads soaked with dilute (700–750 mLD/L) isopropanol. For collecting skin-puncture-derived blood for chemical testing, compounds containing iodine should be avoided (9).

Drying the puncture site. After the puncture site is washed with sterile 70% isopropanol, it must be scrupulously dried with a sterile absorbent pad or sponge. Dryness is essential to avoid hemolysis caused by alcohol or water. Diethyl ether should not be used, because its rapid evaporation cools the skin surface, and it is a fire hazard.

Note: Sterile antiseptic and drying pads must be used, to minimize risks of cross-infection. In no aspect of skin puncture should non-sterile materials be used.

Depth of puncture. For the neonate, the depth of puncture must not exceed 2.4 mm, to avoid penetrating bone (calcaneus) and the risk of osteomyelitis (2, 3). This is based solely on the report by Blumenfeld et al. (4). Two considerations, however, strongly suggest that even 2.4 mm is excessive. In the newborn, the major blood vessels of the heel, located at the dermal–subcutaneous junction, are between 0.35 and 1.6 mm beneath the skin surface. Why should the puncture exceed 1.6 mm? A second factor is the effect of compression on the skin during the puncturing process in decreasing the distance between surface and blood vessel (10).

A study in progress, on measuring skin-surface-to-bone distance in the heel, great toe, and middle finger of infants at necropsy, has provided interesting new data. In four of 14 newborns (ages 6 d, 24.5 h, 24 h, and 14 h; corresponding weights, 957, 1323, 1420, and 1711 g) the respective depths at a selected posterior site on the heel were 2.0, 2.2, 2.2, and 2.1 mm. The other 10 newborns showed distances ranging between 2.7 and 6.6 mm. Measurements are made with the help of a probe 0.4 mm in diameter, consisting of dental wire sharpened on the tip, on which a movable rubber baffle is placed. The baffle allows for smoothing the skin once the puncture to bone is made. The depth of the wire's penetration is measured with a vernier caliper, with the baffle held stationary when the wire is removed (baffle-to-tip distance). The probe leaves no disfiguring marks. Tentatively, one may assume that the minimal distance of 2.4 mm is excessive and needs more study.

Lancets for skin puncture. A suitable lancet's puncturing tip for use on newborns must possess at least two properties:

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3 S/P Collection Tray, American Scientific Products, McGaw Park, IL 60085; Health Mark Industries, 22522 E. Nine Mile Rd., St. Clair Shores, MI 48040; Compact Hematray, Flex Lab Corp., 14251 Harper Ave., Detroit, MI 48213.
4 Reiner CV, Meites S. Children's Hospital, Department of Anatomic Pathology, 700 Children's Drive, Columbus, OH 43205.
(a) appropriate length and (b) appropriate width and thickness, or diameter. Manufacturers have provided lancets proposed by the consensus groups, with tip lengths of 2.4 mm, and these have found universal use. Longer tips are also used. The appropriate width of lancets has not been scientifically addressed. If it is assumed that the depth of puncture is no longer a problem, then what size puncture hole should be made (width and thickness, or diameter)? The wider the puncture tip, the greater the expectation that more blood vessels will be pierced, assuring the maximum volume of blood obtainable with a single puncture. Considering the frequency of multiple punctures per request as well as throughout his/her hospital stay, the newborn requires the shortest possible width and/or diameter. The shortest tip reported in reference [1] had a diameter of 0.5 mm, and this diameter provided the reported 400 µL per puncture (5).

At my request, two special lancets were prepared that had tip lengths of 1.6 ± 0.4 mm, and 1.8 ± 0.4 mm, both with diameters of 0.79 mm. For manufacturing reasons, the 1.8-mm tip was selected for evaluation (Figure 1). Four hundred lancets were tested at each of four pediatric centers. With this reduced tip size, three of the four evaluators who had prior experience with the manufacturer's products highly endorsed the use of the lancet for newborns. There was no apparent diminution in the volume of blood obtained. The fourth laboratory, with no prior experience with this manufacturer's lancet, did not endorse the shortened tip and diameter, having obtained an insufficient volume of blood per puncture. The new product is now commercially available.

Note: The "stabbing" technique used by phlebotomists is often highly variable. When a small tip is used, it is imperative that the full length penetrate the skin at the puncture site. Failure to get the appropriate volume of blood (per puncture) from "healthy" full-term newborns is an indication of poor technique; it is not caused by a defect in the lancet.

Spring-driven devices. Such devices, though offering a solution to the problem of how to obtain uniformity in skin-puncture technique, cannot be recommended, owing to the risks of cross-contamination. However for patients with chronic diseases (e.g., the diabetic) each individual can be provided with a spring-loaded device for personal use.

Follow-up of skin puncture. Once a blood collection is done, and while the patient's foot is held above his heart level, either a sterile gauze pad or cotton swab is pressed to the puncture site until bleeding stops. This critical step may prolong the collection to as much as 10–15 min, sometimes even longer. Adhesive bandages for infants should be avoided because the neonate's skin is sensitive to the adhesive, and because of the potential hazard of the infant's placing it in his mouth and aspirating it.

Blood Collection

Grasp the infant's heel with a moderately firm grip, with your forefinger at the arch of the foot, and your thumb placed well below the puncture site, at the ankle. Make the puncture in one continuous deliberate motion, in a direction perpendicular to the skin surface at the puncture site. The pressure with the thumb is eased and re-applied as drops of blood form and are allowed to flow into the appropriate containers.

For finger puncture, place your thumb either below or above, and well away from, the puncture site. As with the heel, make the puncture in one continuous, deliberate motion, in a direction perpendicular to the skin surface at the puncture site. Again, moderately firm pressure is applied without massage.

The first drop of blood must be assumed to contain an excess of intracellular and interstitial fluid, with surface debris. It is discarded by wiping it away with a dry sterile pad.

When filling capillary collection tubes for pH—blood-gas determinations, maintain the patient's heel at approximately body (horizontal) level throughout the collection to avoid venous stasis (a good practice for clinical chemical samples in general). Hold the collecting tube in a nearly horizontal position and allow it to be filled as long as there is sufficient blood present to cover the tip. If blood is being drawn into the capillary faster than it is replaced, withdraw the tube slightly, moving it back into position when there is sufficient blood present. If air is inadvertently drawn into the tube, discard it and replace it with a new one.

Sealing capillary tubes for blood gases, and mixing with anticoagulant. Once the capillary tube is filled with bubble-free blood, seal one end with "wax" to a depth of about 4 mm, or use a plastic cap.

Note: To mix the sample before sealing, gently invert the capillary tube several times. When the blood is ready for analysis, one end of the tube (with about 2 mm of the blood) is scored, broken, and discarded, or the plastic cap is removed.
A small piece of iron wire, about 5 mm long, is then inserted into the tube. The second end is then sealed to a depth of about 2 mm, or a plastic cap is applied. This should force out some of the wax on the other end. Mixing is then done by moving the iron wire back and forth inside the tube with the help of a magnet.

**Labeling.** At present there is no completely satisfactory technique for labeling containers in a manner that not only is convenient and applicable to the various containers used but also assures foolproof identification of the sample. Two procedures are in vogue: (a) direct inscription of the container and (b) affixing adhesive labels. Capillary tubes may be labeled directly with fine-tipped pens or by wrapping a small adhesive label (flag) around them, or by attaching the label to a test tube into which the capillaries are placed for transport. Adhesive labels (often computer-generated) on which accession numbers are printed, coinciding with and detachable from the request form, are popularly used. Larger containers are more readily labeled by either of the two procedures.

**Containers.** Table 1 summarizes the data provided by the consensus group. Containers are available from several sources not listed here.

<table>
<thead>
<tr>
<th>Table 1. Containers for Blood Collection</th>
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<tr>
<td><strong>Container</strong></td>
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<tr>
<td>Blood gases (pH, pO₂, pCO₂): Naloxon glass collecting pipets, 250-μL volume; lithium heparin; heparinized glass capillary tubes, 100-250 μL.</td>
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<tr>
<td>Caraway glass tubes, 370-μL volume, ammonium heparin.</td>
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<tr>
<td>Electrolytes (CO₂, Cl⁻, K⁺, Na⁺) and general chemistry: Microhematocrit glass capillary tubes 1.1-1.2 mm i.d., 75 mm long, ammonium heparin; other sizes.</td>
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<tr>
<td>Micro sample tubes, polyethylene, 300 μL, lithium heparin; larger sizes (400-550 μL) for general chemistry, with and without heparin.</td>
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<tr>
<td>B-D Microtainer, polypropylene; 600, 700 μL; silicone separator; with and without lithium heparin.</td>
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*Containers listed here are all commercially available.

*Mixing without use of a "flea" is also possible by gently inverting the capillary before sealing.

**Specimen Transport**

Sealed specimens for blood gases are immersed in iced-water (not ice alone) for transport. This should be done immediately after blood collection to minimize the rapid effects of leukocyte and erythrocyte glycolysis. "Zip-lock" plastic bags containing ice water are convenient containers for this procedure.

**Centrifugation of Microsamples**

Table-top, high-speed centrifuges, specifically designed for centrifuging microtubes, are currently used. Attaining forces up to about 10 000–14 000 × g, they separate plasma or serum within 1–2 min. In this respect, they are much more efficient than the slower and bulkier floor centrifuges. The microcentrifuges are a requisite in the pediatric clinical microchemistry laboratory. Small-bore glass capillary tubes are readily spun in special centrifuges. There has apparently been no study of temperature changes within the chamber of the microcentrifuges during centrifugation. Larger centrifuges generate heat during their operation with potential damage to labile substances in blood and other body fluids. A refrigerated microcentrifuge could prove very useful.

**Discussion**

Blood does not flow freely from skin puncture of infants, particularly those who are dehydrated or in shock. With the techniques described here, moderate pressure near the puncture site must be maintained to assure the flow. Drops of blood usually form on the skin surface, one by one. Necessarily, blood is exposed to air when this technique is used, but this is unrelated to the question of which container to use (capillary vs larger-bore tube) and how the container may affect the quality of the sample for blood-gas determination.

While consensus groups have disseminated improved techniques for skin puncture, and manufacturers have provided appropriate equipment for that purpose, there is little uniformity among those designated to perform phlebotomy. A problem existing is one of adequately training personnel for skin puncture. The nine contributors to this article have indicated that phlebotomy is the job of the (a) clinical laboratory, (b) nursing staff, or (c) an autonomous unit. For the "off-hours" (nights, weekends, holidays), three institutions use house-officers (physicians). Obviously there is a continual need to train and update phlebotomists, and to establish standards of quality. One such standard is the incidence of hemolysis in specimens—a reflection of poor technique. For infants older than 13 days, there should be no visible hemolysis in the plasma or serum; for neonates less than 13 days old, visible hemolysis occurs in less than 5% of samples. Poor skin-puncturing technique and improper sample-handling are involved.

We have only lately perceived the lacuna in our factual knowledge of skin puncture. Scientific experiment and perhaps a modicum of consensus will reveal the underlying truth involved in anatomy, compression, skin resistance, the force to penetrate skin, and the proper depth and, more importantly, the width of the lancet tip.

More information is also needed about the economy of skin puncture. To improve our practices, we need data on

* Beckman Microfuge 11,12,Airfuge; Eppendorf 5414; Fisher 59A,2235C; IEC Micro-MB; American Biofuge B.
the relative use and frequency of skin puncture during the patient's hospital stay, its age-related component, how its incidence relates to venipuncture, and on blood sampling from indwelling catheters.

Although non-invasive diagnostic techniques for measuring blood and tissue constituents have made progress, it appears unlikely that the next decade will bring a diminution in the necessity for skin puncture. Indeed, in the U.S.A., the use of rapid, ultramicro, automated blood tests has made possible mass and group screening of skin-puncture-derived blood for neonatal genetic disorders, and for glucose and cholesterol. Other analytes will be added. Based on factual knowledge, greater quality in skin-puncture practices and the equipment used will contribute positively toward newer measures of preventive medicine.

References

A Multi-Factor Experimental Design for Evaluating Random-Access Analyzers

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A multi-factor experimental design for evaluating random-access analyzers has been developed and tested for the Ciba Corning "550 Express" random-access analyzer. The 12-sample design estimates imprecision, slope, nonlinearity, linear drift, and reagent carryover to the next assay. The design was constructed so that estimates of the factors' effects are almost entirely uninfluenced by each other. Use of the design is illustrated by an example in which reagent-to-assay carryover was pinpointed as an apparent cause of high imprecision. This led to a modification of the analyzer such that carryover was insignificant. The appendix contains a 27-sample design that provides additional estimates. Software to perform such calculations is available on request.

We describe a multi-factor design developed for evaluating a random-access analyzer. An advantage of multi-factor designs compared with one-at-a-time designs is that one can efficiently estimate factor effects simultaneously. The 12-sample design estimates imprecision, slope relative to reference values, linear drift, reagent carryover to next assay, and nonlinearity of instrument response to analyte concentra-

1 Ciba Corning Diagnostics Corp., 63 North St., Medfield, MA 02052.
2 Ciba Corning Diagnostics Corp., Gilford Systems, 132 Artino Street, Oberlin, OH 44074.
3 24 Burroughs Street, Jamaica Plain, MA 02130.
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