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Comparative evaluation of newborn bloodspot specimen cards by experienced laboratory personnel and by an optical scanning instrument

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ABSTRACT

A major factor in determining the suitability of a dried blood spot (DBS) specimen is the subjective nature of evaluation by laboratory personnel. Using newborn screening DBS specimen cards as they were submitted to a public health NBS program, we conducted a systematic pilot study of DBS evaluation by multiple experienced laboratory personnel (ELP) and by an automated optical scanning instrument (OSI) (CardScan (tm), BSD Robotics). OSI confirmed the satisfactory status of all newborn DBS specimen cards that passed initial review by the first ELP. Among the questionable cards selected for further review, 58% passed multiple ELP consensus assessment, and 62% passed OSI evaluation. The overall agreement between ELP and OSI was 86%. Among questionable specimen cards, ELP and OSI were more strongly correlated when multiple ELP assessment was unanimous. We conclude that subjective assessment by ELP is essential and that OSI evaluation is a useful adjunct when ELP assessment does not reach consensus. OSI further allows the selection of optimal locations for punching DBS from unsatisfactory or questionable specimens, optimizing the quality of interim analyses that may be conducted while repeat specimens are being collected. Instrument evaluation of specimen cards would also be valuable as an independent reference method for training laboratory and specimen collection personnel. OSI technology merits further studies to confirm and extend our findings.

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1. Introduction

The first critical step in newborn bloodspot screening (NBS) is the collection of satisfactory dried blood spot (DBS) specimens. High quality results cannot be ensured with low quality specimens. From the origin of public health NBS [1], the criteria for discriminating satisfactory from unsatisfactory DBS specimens has been based on visual inspection and subjective assessment by experienced laboratory personnel (ELP) [2–4]. However, no systemic study of variance in ELP ability to distinguish acceptable from unacceptable specimens has been reported. Moreover, instruments have recently become available that evaluate DBS and select optimal areas for obtaining punches for analysis, but these instruments have not been compared to ELP assessment in a systemic study. To address this gap, we conducted a systematic pilot study of variance in the ELP assessment of newborn DBS specimens submitted to a public health laboratory and compared it to an assessment by an optical scanning instrument (OSI).

2. Materials and methods

2.1. DBS reference specimens

DBS reference specimens were prepared on collection cards used by the Newborn Screening Quality Assurance Program (NSQAP) at the US Centers for Disease Control and Prevention (CDC). DBSs simulating acceptable newborn specimens were prepared by applying 75 μL of normal adult blood with hematocrit adjusted to 50% [2]. DBSs containing an excessive blood volume were prepared by applying two aliquots of 75 μL to the same spot, and DBSs with a deficient blood volume were prepared by applying 10 μL per spot. DBSs containing a deficient red blood cell content were made by applying 75 μL of normal adult blood with the hematocrit reduced to 27%. Misshapen DBSs were made by moving the pipette tip while dispensing 75 μL of normal adult blood. DBSs were also made with 75 μL of normal blood lysed

Abbreviations: CDC, Centers for Disease Control and Prevention; DBS, dried blood spot; ELP, experienced laboratory personnel; GDPH, Georgia Department of Public Health; OSI, optical scanning instrument.

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by freezing. All DBSs were allowed to dry at room temperature for a minimum of 3 h before evaluation.

2.2. Newborn DBS specimen collection cards

The study was conducted on newborn DBS specimen cards submitted to the Newborn Screening Laboratory at the Georgia Department of Public Health (GDPH) for routine screening. Each DBS specimen card contained either 5 or 6 heelstick blood spots.

2.3. Evaluation by experienced laboratory personnel

For routine operations, all newborn screening DBS specimen cards are initially evaluated in the Accessioning Section of the GDPH Laboratory. Any specimen cards deemed to be questionable are then reviewed by the Newborn Screening Laboratory manager or a Newborn Screening Unit supervisor, who [1] makes the final decision whether to grade the overall card as acceptable, and [2] determines whether there are preferred or unacceptable blood spots on the card. In this program, a total of nine 3-mm punches are needed for initial testing. Additional punches are required for any necessary repeat or reflex testing. For this reason, a specimen is considered unsatisfactory if it does not have at least two acceptable blood spots each of which can produce 5–6 punches.

For this study, 77 DBS specimen cards that were initially deemed questionable by one ELP in the Accessioning Section were independently reviewed by three other ELP in the Newborn Screening Laboratory. Each spot on each card was rated as 0 (unacceptable), 1 (marginal), or 2 (acceptable), and the entire specimen card was then graded as either satisfactory or unsatisfactory. An additional 100 DBS specimen cards that were initially graded as satisfactory in the Accessioning Section were also subjected to OSI assessment.

2.4. Evaluation by optical scanning instrumentation

An OSI (CardScan (BSD Robotics, Brisbane, Australia)) provided for evaluation was first installed at CDC by one of the authors (DG), and parameters were adjusted using DBS reference specimens. Blank NSQAP DBS collection cards were used to create a template map by which the instrument located the individual blood spots on each card. Instrument settings were adjusted to identify at least 20 locations among all DBS on a specimen card from which suitable 3 mm punches could be obtained. OSI evaluation parameters were optimized to discriminate acceptable, marginal, and unacceptable DBS and to identify acceptable punch areas within acceptable and marginal DBS. The OSI was then transferred to the GDPH laboratory, where new template maps were created for the GDPH DBS collection cards. Some of the OSI parameters originally set at CDC were refined by GDPH for evaluating actual newborn DBS (Table 1).

Prior to each use, an instrument system calibration was completed using the CardScan Calibration Card. The calibration process adjusted the optical source light-emitting diodes (LEDs) to achieve pre-set values for the Transmission Target and the Compensation Target. The OSI evaluation algorithm (Fig. 1) includes area, circularity, convexity, and consistency. The area criterion evaluates whether the DBS is in the proper overall size range. Circularity and convexity are used to determine that the blood spreads symmetrically from the application point. Consistency evaluates the uniformity of blood dispersion.

A clear plastic sleeve was placed over each DBS specimen card to ensure that the card was flat while being scanned. Each scan required about 3 s. The results appeared on the computer screen with a scanned image of the DBS card showing colored circles superimposed on acceptable locations for punching 3 mm discs (Fig. 2). Specimen cards with at least 20 such locations were considered satisfactory. About 100 blood spot cards could be scanned in an hour.

Table 1
Optical scanning evaluation parameters.

	Default[a]	CDC[b]	GDPH[c]
Area	50–250 mm[b]	50–200 mm[b]	75–200 mm[b]
Circularity			
Pass	60%	50%	60%
Intermediate	45%–60%	43%–50%	45%–60%
Fail	<45%	<43%	<45%
Convexity			
Pass	>90%	>50%	>90%
Intermediate	80%–90%	43%–50%	80%–90%

[a] Default = original instrument settings.
[b] CDC (Centers for Disease Control and Prevention) = settings established using DBS reference materials.
[c] GDPH (Georgia Department of Public Health) = settings refined for use with newborn blood spots.

3. Results

3.1. CDC DBS reference specimen cards

All specimen cards containing DBS made with the 75 μL of normal blood were evaluated as satisfactory by OSI, with a distribution of acceptable punch locations on each DBS. All of the specimen cards made with either 150 μL or 10 μL of blood were evaluated as unsatisfactory, with no suitable punch locations identified on any DBS.

3.2. Newborn DBS specimen cards initially assessed as satisfactory

Of the 100 DBS specimen cards initially assessed as satisfactory by a single ELP in the Accessioning Section, all cards (100%) were found to be satisfactory by OSI evaluation.

3.3. Newborn DBS specimen cards initially assessed as questionable

Of the 77 cards initially assessed as questionable in the Accessioning Section, 45 (58%) were subsequently re-assessed as satisfactory: 33 (43%) by all three ELP, and 12 (16%) by two of three ELP. Among the 32 that were re-assessed as unsatisfactory, 19 (25%) were rejected by all three ELP, and 13 (17%) by two out of three ELP (Fig. 3). Overall, the complete ELP consensus was reached on 52 (68%) of the questionable specimen cards, and only partial agreement on 25 (32%).

OSI evaluation of these 77 questionable cards found 48 (62%) to be satisfactory, 19 (25%) to be unsatisfactory, and 10 (13%) to be questionable. When these OSI-questionable cards were grouped with those evaluated as unsatisfactory, the overall agreement between OSI and ELP was 52/77 (67%). Among all 177 newborn DBS specimen cards that were evaluated by OSI, 152 (86%) agreed with ELP assessment.

The categorical agreement between ELP and OSI assessment depended on the extent of ELP consensus (Fig. 4). The overall agreement between ELP assessment and OSI evaluation was higher on specimens with full consensus by ELP (36/52, 69%) than on specimens with partial consensus by ELP (11/25, 44%).

4. Discussion

The utility and acceptance of DBS specimens have increased over the last 50 years primarily because of concerted efforts to control, minimize, and eliminate analytical variations [1]. The filter paper matrix for DBS specimens gives the appearance of a simple sample for analysis, however, many unanticipated complexities are contained within the sample that require control to achieve quality analytic performance.

The first article on NBS for phenylketonuria (PKU) using the Guthrie DBS Bacterial Inhibition Assay (BIA) [5] was initially rejected because