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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 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 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.

Keywords

Newborn bloodspot screening; automation; evaluation; specimen quality; training; dried blood spots

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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) [2,3,4]. 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 [5], but these instruments have not been compared to ELP assessment in a systemic study. To address this gap, we evaluated variance in ELP assessment of newborn DBS specimens submitted to a public health laboratory and compared it to 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). DBS simulating acceptable newborn specimens were prepared by applying 75uL of normal adult blood with hematocrit adjusted to 50% [2]. DBS containing an excessive blood volume were prepared by applying two aliquots of 75 ul to the same spot, and DBS with a deficient blood volume were prepared by applying 10 uL per spot. DBS containing a deficient red blood cell content were made by applying 75 uL of normal adult blood with the hematocrit reduced to 27%. Misshapen DBS were made by moving the pipette tip while dispensing 75ul of normal adult blood. DBS were also made with 75uL of normal blood lysed by freezing. All DBS were allowed to dry at room temperature for a minimum of three hours 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 3mm punches could be obtained. OSI evaluation parameters were optimized to discriminate acceptable, marginal, and unacceptable DBS and to identify acceptable punch areas within acceptable amd 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 (LED) to achieve pre-set values for the Transmission Target and the Compensation Target. The OSI evaluation algorithm (Figure 1) includes area, circularity, convexity, and consistency.

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

3. Results

3.1 CDC DBS Reference Specimen Cards.

All specimen cards containing DBS made with the 75 uL 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 uL or 10 uL 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 (Figure 3). Overall, 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 (Figure 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) (6) was initially rejected because another contemporary study using the same approach found a high number of false-positive results (7). This report recommended against using the Guthrie test for PKU screening. Guthrie later noted (8) that a different source of filter paper, which might not have been absorbent enough for uniform spotting, had been used by the other investigators, probably accounting for their high false-positive rate. Guthrie developed some minimal criteria for production of uniform DBS specimens: the DBS should appear similar on both sides of the paper, should be a consistent diameter (12mm to 13mm) (2) and should be located in a manner to allow easy punching with a standard paper-hole puncher (8).

In late 1970’s, with the introduction of quantitative screening for congenital hypothyroidism using thyroxine (9) and Thyroid Stimulating Hormone (10) assays, the consistent and reproducible performance of the filter paper matrix surfaced as a critical concern for these assays (11). This observation led to the introduction of a CLSI approved standard in 1982, which contributed substantially to the quantitative consistency of filter paper both within and among lots of paper produced by the manufacturer and guided the extensive applications of this matrix across a variety of fields. This consensus standard certified the performance for blood absorption and lot-to-lot consistency of the paper matrix (2). These contributions

enhanced the performance and minimized the contribution of the matrix to the sample for analysis, but did not minimize or control the variables contributed by the actual blood application to the paper matrix.

Identification of unsatisfactory newborn DBS specimens is critical to the reliability of NBS. A DBS punch should contain analyte concentrations that reflect whole blood concentrations, which requires homogenous distribution of the original blood specimen. Depending on the analyte and the disorder, punches that contain varying amounts of dried blood can lead to false positive or false negative screening results. The visual appearance of DBS by ELP provides the usual basis for identifying unsatisfactory specimens. Evaluation by an OSI replaces human visual assessment with the objective measurement of transmitted light throughout the bloodspot. Parameters of the OSI can be adjusted to accomplish a more liberal or a more conservative assessment so as to be consistent with the policy of the NBS program. Once those parameters are set, OSI assessment is an objective process, whereas ELP assessment is invariably subjective.

Parameters for OSI evaluation were first established using NSQAP DBS reference materials made with the proper volume of whole blood. In addition, some DBS were intentionally made with insufficient or excessive blood volumes, all of which were evaluated as unsatisfactory by OSI criteria.

The complete agreement between ELP and OSI on newborn DBS specimen cards initially rated as suitable for analysis shows that the initial triage was highly sensitive to identifying specimens which might be unacceptable. However, agreement between ELP and OSI on the status of questionable DBS cards was much lower. Moreover, disagreement between ELP and OSI was associated with greater variance within multiple ELP. This finding makes it clear that that some specimens present intrinsic challenges to assessment, be it objective or subjective.

We conclude that OSI assessment is a promising adjunct for judging the acceptability of newborn DBS specimens. Since it is not affected by evaluator bias or fatigue, OSI may be especially useful for training and in resolving discrepant evaluations among ELP. The ability of OSI to identify preferred punch areas could optimize interim analyses while awaiting a second specimen when a recollection is necessary.

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

REFERENCE

1. Hannon WH, Therrell BL, Overview of the History and Applications of Dried Blood Samples. in Lee MS and Wenkui L (Eds.), Dried Blood Spots Application and Techniques, John Wiley & Sons Inc. Hoboken, New Jersey 2014, pp. 3–15 in press.
2. Clinical and Laboratory Standards Institute (CLSI). Blood Collection on Filter Paper for Newborn Screening Programs; Standard—Sixth Edition. CLSI document NBS01-A6 (ISBN 1-56238-883-5 [Print]; ISBN 1-56238-884-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, July 2013.
3. Sherwin J, Web Conference on Unsatisfactory Newborn Screening Specimens: Interpretations, Studies and Current Trends. PowerPoint Presentation, 05/17/2005. www.cdc.gov/labstandards/nsqap_conferences_2005.html.
4. Ontario Newborn Screening Program: Blood Spot Collection 2009. www.newbornscreening.on.ca.
5. Luminex CardScan™ Software Installation and User Manual. © Luminex Corporation 2013.
6. Guthrie R, Blood Screening for Phenylketonuria, Journal of the American Medical Association. (1961) 178: 863.
7. Scheel C, Berry HK, Comparison of Serum Phenylalanine Levels with Growth in Guthrie's Inhibition Assay in Newborn Infants. Journal of Pediatrics (1962) 61: 610–616.
8. Guthrie R, Susi A, A Simple Phenylalanine Method for Detecting Phenylketonuria in Large Populations of Newborn Infants. Pediatrics (1963) 32: 338–343. [PubMed: 14063511]
9. Dussault JH, Laberge C, Thyroxine (T4) Determination in Dried Blood by Radioimmunoassay: a Screening Method for Neonatal Hypothyroidism. Union Medicale du Canada (1973) 102: 2062–6064.
10. Walfish PG, Evaluation of Three Thyroid-Function Screening Tests for Detecting Neonatal Hypothyroidism. Lancet (1976) (1) 7971: 1208–1210.
11. Mitchell ML, Improved Thyroxine Radioimmunoassay, for Filter Paper Discs Saturated with Dried Blood. Clin Chem (1976) 22: 1912–1914. [PubMed: 975555]

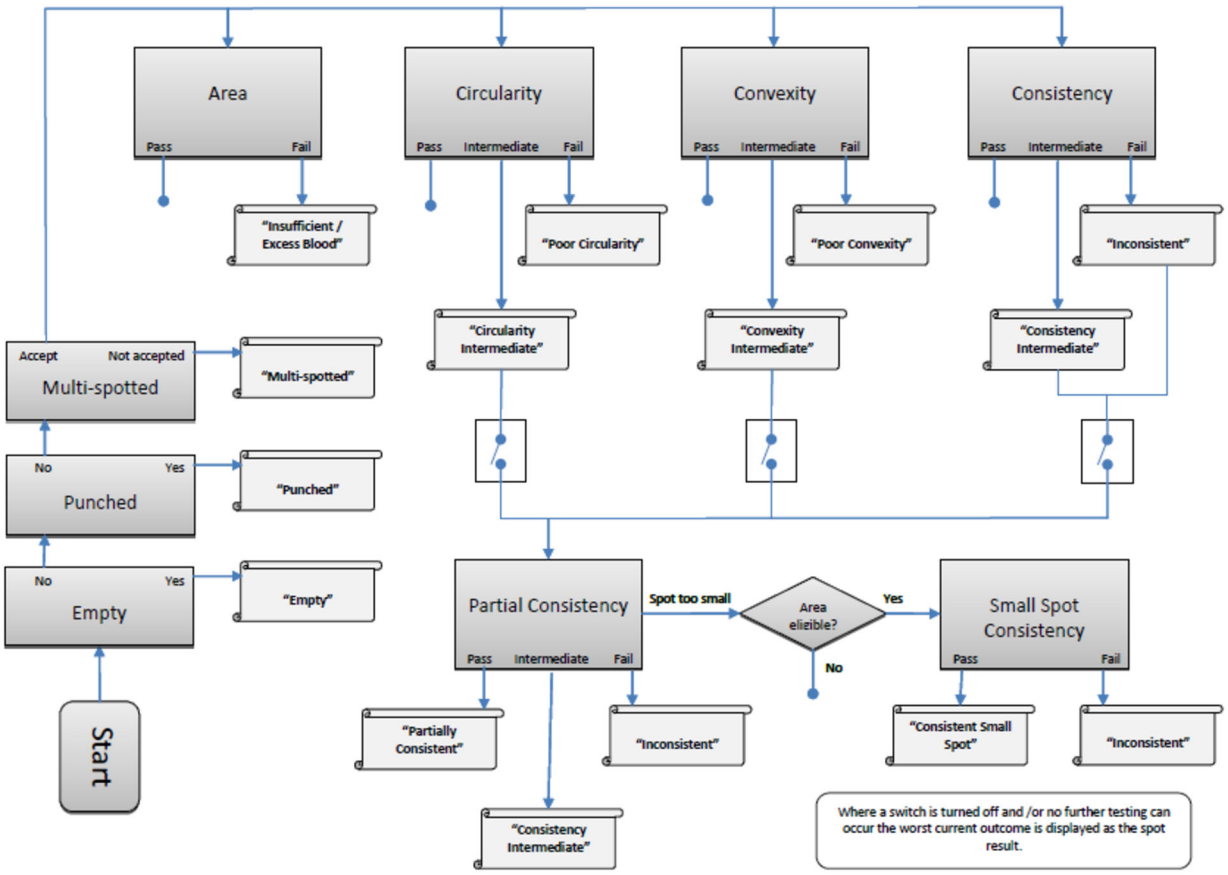

Figure 1. Optical Scanning Instrument Dried Blood Spot Evaluation Algorithm

Flow chart for the Cardscan Dried Blood Spot Scan Instrument showing the parameters and pathways for evaluation.

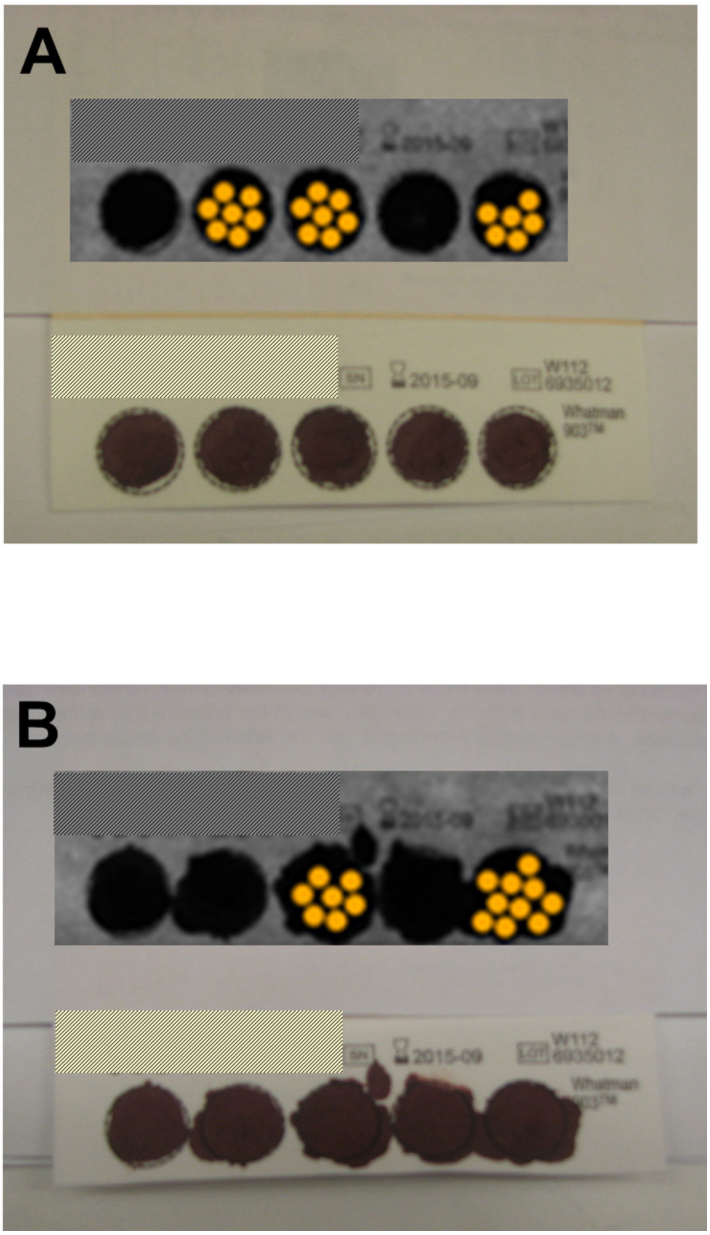

Figure 2. Newborn DBS Specimen collection cards and Optical Scanning Instrument (OSI) Scanned Images

A. OSI instrument found 20 acceptable punch areas in 3 DBS, the minimum required for an acceptable evaluation by laboratory-designated criteria.

B. The OSI instrument found only 17 acceptable punch areas in 2 DBS, resulted in an unacceptable evaluation. The remaining 3 DBS did not have any acceptable punch locations.

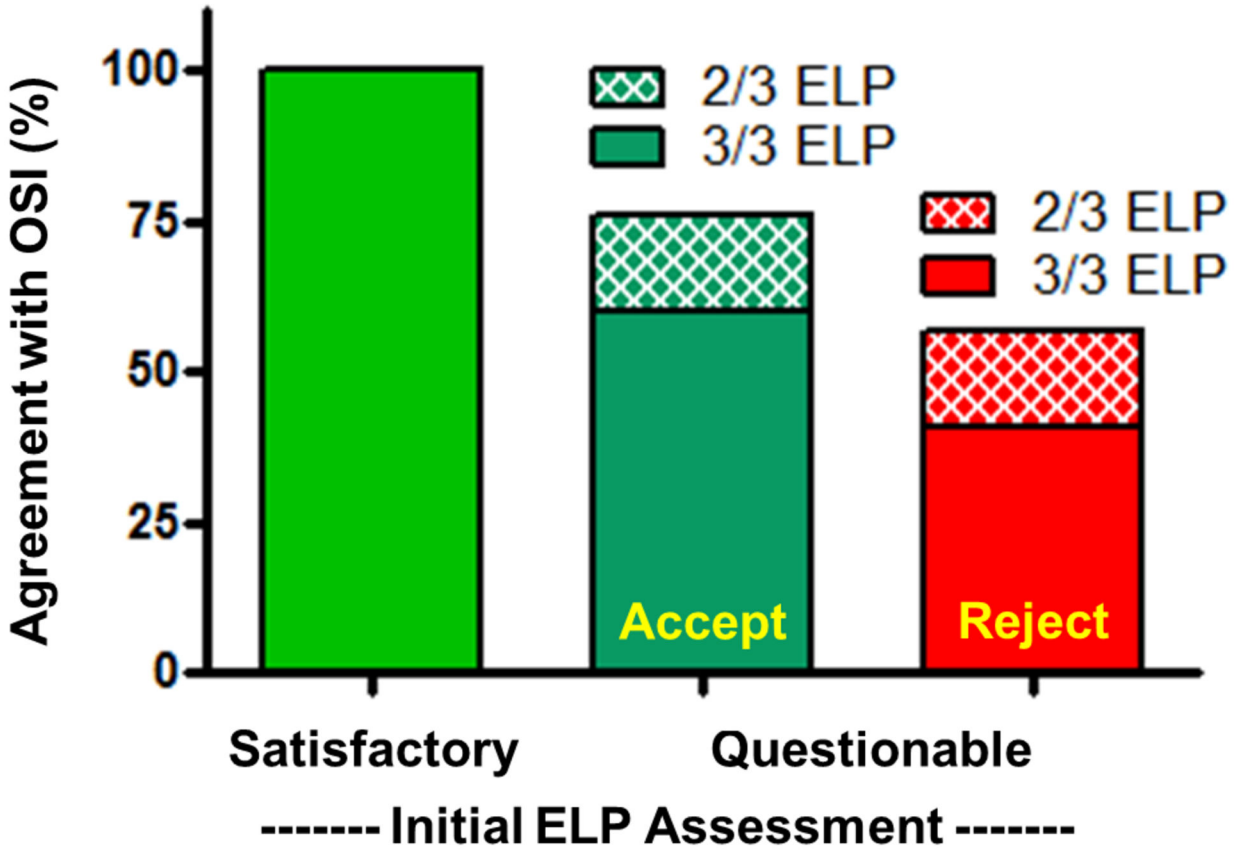

Figure 3. Overall Agreement between Evaluation by an Optical Scanning Instrument (OSI) and Assessment by Experienced Laboratory Personnel (ELP)

The first bar shows 100% agreement between OSI and ELP for 100 DBS cards rated as "satisfactory" in the initial ELP assessment. The second bar represents 76% agreement for 45 cards initially rated as questionable and subsequently assessed as satisfactory by at least two of the three ELP. The third bar represents 57% agreement among the 32 cards initially assessed as questionable and subsequently assessedas unsatisfactory by at least two of the three ELP. OSI questionable results were considered the equivalent of unsatisfactory.

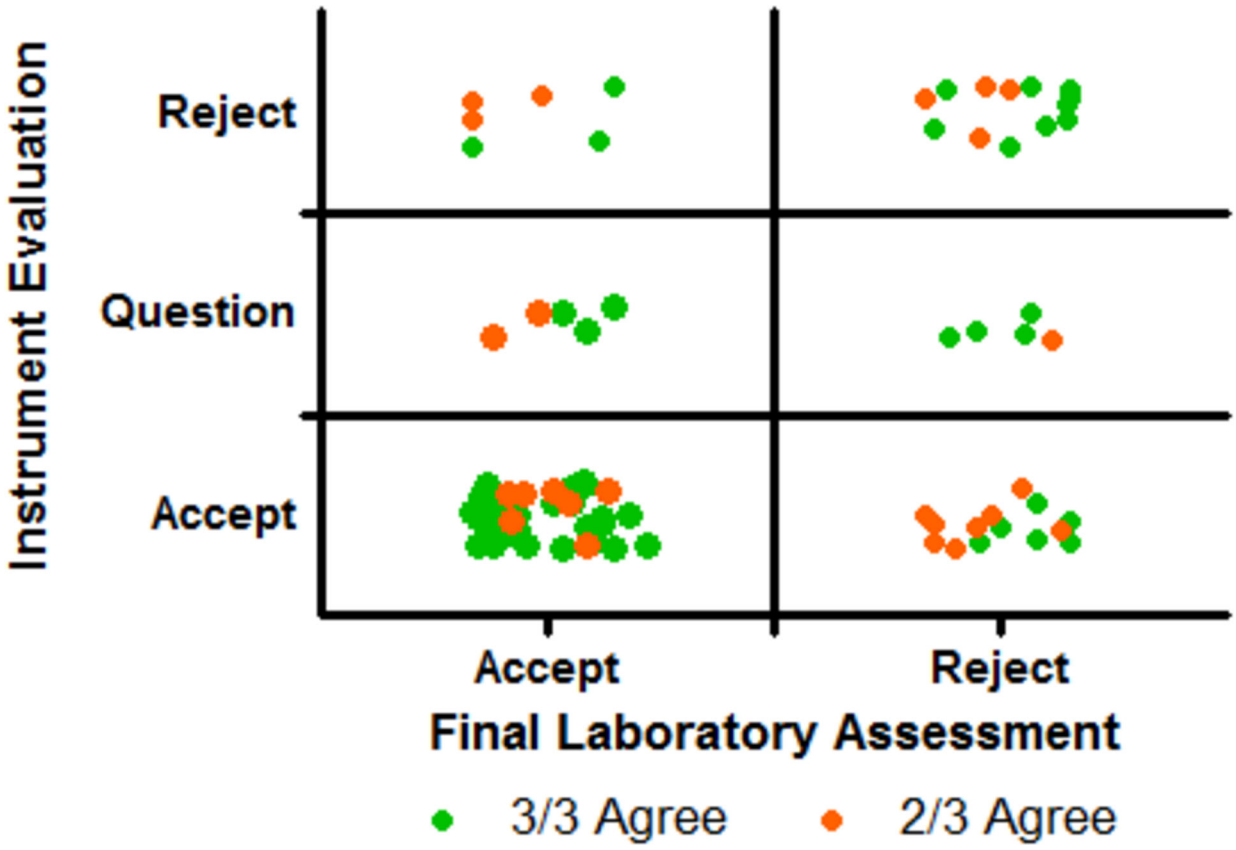

Figure 4. Categorical Agreement between Evaluation by an Optical Scanning Instrument (OSI) and by Experienced Laboratory Personnel (ELP)

Date from Figure 2 are depicted in their individual categories, including the questionable OSI evaluation category.

Table 1.

Optical Scanning Evaluation Parameters

	Default[1]	**CDC**[2]	**GDPH**[3]
Area	50–250mm^2	50–200mm^2	75–200mm^2
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%

[1] Default = original instrument settings

[2] CDC (Centers for Disease Control and Prevention) = settings established using DBS reference materials

[3] GDPH (Georgia Department of Public Health) = settings refined for use with newborn blood spots