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Glue for Manufacturing Heel Prick Filter Cards Does Not Interfere with the Measurement of Analytes for Newborn Screening

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Abstract: Population-based newborn screening (NBS) using blood collected and dried on filter paper was developed in the 1960s and remains the international standard for NBS programs. Glue, used in the manufacture of dried blood collection cards, may present a source of contamination and is often considered as a possible cause of anomalous results in routine screening. Our study evaluates this potential contamination on NBS analyses. EBF#1003 glue was blotted onto dried blood collection cards made of Whatman grade 903 filter paper (Whatman#903) and adult whole blood was pipetted onto the dried glue blots. In addition, blank glue blots (i.e., no blood) and dried blood spots (DBSs) in the absence of glue were prepared. The DBSs and blank samples were run in duplicate as routine samples for NBS. DBS absorption time and diameter, the effect of glue on measurements (concentrations and variation) were assessed. DBS absorption time and shape were equivalent for DBSs prepared in the absence and presence of undiluted glue. DBS diameter increased when prepared in the presence of glue. When EBF#1003 was diluted prior to use, DBS absorption time increased, and DBSs were non-uniform. Glue, diluted or not, did not produce measurements above the established Limit of Detection (LOD) for all assays used in the current Dutch screening programme. For all analytes with concentrations in the quantifiable range, contamination with glue had no effect on measurement variation, as it appeared equivalent to variation in untreated DBSs. Our data show that, in the unlikely event of contamination of Whatman#903 with EBF#1003, there is no effect on the measured concentration of analytes.

Keywords: newborn screening; NBS; Whatman#903; EBF#1003 glue; contamination; interference

1. Introduction

The Dutch neonatal screening (NBS) programme, started in 1974, currently screens for 19 diseases: conducted in five laboratories, it is available to all babies born in The Netherlands and uses the filter paper card often referred to as a "Guthrie Card". As with all methods of blood collection, there is associated imprecision and variability with the use of filter papers, for example, the variable distribution of analytes in the dried blood spot (DBS) (the "chromatography effect"), blood absorption time, contamination, and the filter paper matrix, which can all have an influence on the recovery of analytes [1,2]. For this reason, approved standards for the manufacture of filter paper for NBS purposes are available [3]. Transitions between filter paper types and filter paper lots should be minimized, but are inevitable. In 2016, the heel prick filter card used in the Dutch NBS programme, PerkinElmer grade 226 filter paper (PerkinElmer#226), was replaced by Whatman grade 903 filter paper (Whatman#903). A verification study has been conducted in our laboratory to assess the effect this change would have on NBS analysis; the results illustrate that the two filter papers are essentially

equivalent, an observation which concurs with a previous study comparing PerkinElmer#226 and Whatman#903 [4]. Glue, used in the manufacture of heel prick cards, anecdotally has been considered a cause for unexplained high measurement levels in NBS and is assumed to interfere with the measurements of analytes. However, there are no published studies that we are aware of that evaluate this effect. As part of the verification study for Whatman#903, the manufacturer of the cards uniquely enabled us to evaluate the effect of glue contamination on NBS analysis and to state that the chances of glue contamination in the production process are extremely small. Below, we present the effect of glue on the measurements of analytes employed in Dutch newborn heel prick screening, tested as glue on filter paper and in combination with DBSs, evaluating multiple assays and multiple analytes.

2. Materials and Methods

Whatman#903 and the EBF#1003 glue used in the manufacture of Whatman#903 were provided by Eastern Business Forms, Inc., Mauldin, SC, USA.

2.1. Preparation of Glue

In order to assess whether any observed effects are dependent on the concentration of glue, three concentrations of glue were prepared to provide a range of quantifiable glue concentrations: undiluted, 1:1 diluted with demineralized water, and 1:4 diluted with demineralized water.

2.2. Preparation of Glued Filter Paper

For each of the three concentrations of glue: 15 µL of glue was pipetted onto a plastic template (12 mm × 22 mm) and evenly spread using a plastic spatula. The template was blotted onto the filter paper and held in place for 5 s before removing.

2.3. Preparation of DBSs on Filter Paper Treated with Glue

Adult whole blood was collected in a lithium heparin vacutainer and 50 µL was pipetted on filter paper treated with glue for each of the glue concentrations. DBSs were also prepared on filter paper not treated with glue, "untreated filter paper". The filter paper was dried on a horizontal, non-absorbent surface for at least 3 h at ambient temperature [5]. For the duration of the study, the filter paper was stored at ambient temperature.

Absorption times and absorption diameters were recorded for each glue concentration. Absorption time was measured with a stopwatch, which was started when the blood touched the filter paper and stopped when any shine from the liquid blood was no longer visible on the surface. Absorption diameters were measured at the widest diameter [6].

2.4. Analysis of DBSs

Punches (3.2 mm) were obtained from the various combinations of DBSs and glue-treated filter paper and run in duplicate as routine samples for all routine NBS analyses conducted in the Dutch NBS programme (see Table 1 for overview), assessing the concentration of a total of 28 analytes. Due to practical constraints, Total Galactose (TGAL) and Thyroid Stimulating Hormone (TSH) analyses were not included in this study. Interference and carryover were also assessed.

The presented results are the DBS absorption characteristics (Table 2) and concentrations of analytes, when exceeding the Limit of Detection (LOD) values internally applied by our laboratory (Table 3). Variation is expressed as standard deviation of the mean (SD) and as coefficient of variance (%CV). Measured analyte concentrations of DBSs prepared on filter paper treated with glue are also expressed as a percentage of concentrations in DBSs on untreated filter paper (Table 4).

Table 1. Analytical methods employed and analytes measured within the Dutch Newborn Screening (NBS) programme, and for which the effect of glue was evaluated (RIVM: reference laboratory for neonatal screening).

Analytical Instrument	Analyte
Genetic Screening Processor (GSP) (PerkinElmer, Turku, Finland)	17α-OH Progesterone (OHP) Thyroxine (T4) Thyroid Stimulating Hormone (TSH) * Galactose-1-Phosphate Uridyltransferase (GALT) Immunoreactive Trypsinogen (IRT)
Waters Quattro Micro tandem mass spectrometer (MS/MS) with Neobase Non-derivatized MS/MS assay with Neobase Succinylacetone Assay Solution (PerkinElmer)	Amino Acids (Leu, Phe, Tyr, Val) and acylcarnitines (C0, C2, C5, C5DC, C6, C8, C10, C10:1, C14, C14:1, C14:2, C16, C16:1, C16:1-OH, C18:1-OH, SA)
HPLC (Bio-Rad VARIANTnbs Sickle Cell Program)	Hemoglobins (Hb)
Anthos-Zenyth 340r photometer (filters 540/690) with Bio-Rad Quantase kit	Biotinidase (BIOT)
Anthos-Zenyth 340r photometer (filters 570/690) with Bio-Rad Quantase kit	Total Galactose (TGAL) *
Anthos-Zenyth 340r photometer (filters 450/620) with Monobind Accubind ELISA	Thyroxine binding globulin (TBG)
PerkinElmer Delfia 1234 fluorometer with dynabio MucoPAP-F kit	Pancreatitis Associated Protein (PAP)

* TSH and TGAL were not evaluated in this study.

3. Results

3.1. Absorption time and Absorption Diameter

The absorption times of DBS specimens prepared in the absence and presence of undiluted glue are equivalent. DBS diameter shows an increase of 17% when prepared in the presence of undiluted glue (Table 2). An increase in absorption time and absorption diameter of approximately 80% and 20%, respectively, is associated with dilution of the glue with demineralized water (1:1 and 1:4 dilutions). The observed increases in absorption time and absorption diameter are independent of the level of dilution. The DBSs prepared in the presence of diluted glue (at both concentrations) were non-uniform in shape and non-uniform in blood distribution on the side of blood application, but appeared uniform in color on the reverse side of the filter paper.

Table 2. Absorption time and absorption diameter for dried blood spots (DBSs) prepared in the presence and absence of glue. Please check and confirm.

Absorption Characteristics	DBS-No Glue	DBS-Glue	DBS-Glue (1:1)	Glue (1:4)
Absorption time (s)	4.1 ± 0.7 (16.2)	4.2 ± 0.3 (7.7)	7.4 ± 1.0 (13.9)	7.8 ± 0.5 (6.8)
Diameter (mm)	11.5 ± 0.6 (5.0)	13.5 ± 0.6 (4.3)	14.0 ± 0.0 (0.0)	14 ± 0.0 (0.0)

Note: data presented is mean ± SD (%CV) of n = 4 individual measurements.

3.2. Measurements of Analytes in Filter Paper Treated with Glue

Measurements of analytes in filter paper treated with glue, but in the absence of DBSs, are presented in Table 3. For almost all analytes, measurements in filter paper treated with glue were either below the LOD established within our laboratory or below the LOD as stated in the kit insert of the assay (PAP)—otherwise, there was no measurement signal (Hb peaks). For analytes where an LOD was not defined, measured concentrations were close to zero. With the exception of SA, none of the measurements in glue-treated paper produced values above those in untreated filter paper. For SA, measurements in paper with glue were almost equal to those in untreated filter paper because

of a high LOB (a result of high variation at low measured concetrations of SA). For GALT, quenching of the signal by hemoglobin in the dried blood spot is essential, so no reliable measurement signal was obtained in the absence of DBSs. No relation was observed between measurements and concentration of the glue for any of the analytes available (Table 4).

Table 3. Concentrations of analytes in untreated filter paper (no DBSs) and filter paper treated with glue (no DBSs) †.

Analyte	Untreated	Glue Undiluted	Glue Diluted 1:1	Glue Diluted 1:4	Laboratory-Established LOD
OHP	<LOD	<LOD	<LOD	<LOD	0.5
T4	ND	ND	ND	ND	10
TBG	<LOD	<LOD	<LOD	<LOD	Not established
BIOT	2 + 1.4	2 + 1.4	2 + 1.4	2 + 1.4	Not established
GALT	-	-	-	-	Not established
Hb-FAST	No peak	No peak	No peak	No peak	Not established
Hb-F	No peak	No peak	No peak	No peak	Not established
Hb-A	No peak	No peak	No peak	No peak	Not established
Hb-E	No peak	No peak	No peak	No peak	Not established
IRT	<LOD	<LOD	<LOD	<LOD	10
PAP	<LOD assay	0.1 + 0	0.0 + 0.0	<LOD assay	Not established
Leu	<LOD	<LOD	<LOD	<LOD	10
Phe	<LOD	<LOD	<LOD	<LOD	10
Tyr	<LOD	<LOD	<LOD	<LOD	40
Val	<LOD	<LOD	<LOD	<LOD	15
C0	<LOD	<LOD	<LOD	<LOD	2
C2	<LOD	<LOD	<LOD	<LOD	1.5
C5	<LOD	<LOD	<LOD	<LOD	0.08
C5DC	<LOD	<LOD	<LOD	<LOD	0.25
C5-OH	<LOD	<LOD	<LOD	<LOD	0.25
C6	0 + 0	0 + 0	0 + 0	0 + 0	Not established
C8	<LOD	<LOD	<LOD	<LOD	0.06
C10	<LOD	<LOD	<LOD	<LOD	0.04
C10:1	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	Not established
C14	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	Not established
C14:1	<LOD	<LOD	<LOD	<LOD	0.1
C14:2	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	Not established
C16	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	Not established
C16:1	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	Not established
C16:1-OH	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	Not established
C18:1-OH	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	Not established
SA	<LOD	<LOD	<LOD	<LOD	1.0

Note: TSH and TGAL data not determined; † Units in blood: OHP, T4, TBG, nmol/L; BIOT, % c.f. daily mean; GALT, U/dL; IRT, µg/L; Leu, Phe, Tyr, Val, C0, C5-OH, C8, C14:1, C16:1-OH, µmol/L.

3.3. Interaction of Glue with DBSs

To assess the interaction of glue with the measurements of analytes in punches from DBSs, DBSs were prepared on filter paper treated with glue and analyzed. Results from selected analytes, for which measured concentrations were in the quantifiable range (and still reflecting the range of analytes and methods used within the screening programme), are presented (Table 4).

Variation in duplicate measurements was comparable to the variation obtained with DBSs on untreated filter paper for all analytes and, with the exception of OHP, was mostly well below the highest variation observed, 22% (Table 4). The measured concentration of OHP in DBSs, prepared in the presence of undiluted glue and glue diluted 1:4 with deionized water, is more than twice as high as the measured concentration of OHP reported for a DBS prepared in the absence of glue.

Again, with the exception of OHP, for all the analytes and for all concentrations of glue, the measured analyte concentrations were comparable to measurements using DBSs on untreated filter paper, as illustrated by the percentages in italics in Table 4: measurements as expressed as a percentage of the measurement in DBSs on untreated filter paper were all between 64% (C14:1, filter paper treated

with glue diluted 1:4) and 129% (BIOT on filter paper treated with glue diluted 1:1) but were generally around 100%, with no obvious indication of a relation with the concentration of glue.

Table 4. Concentrations of analytes determined from DBSs prepared on filter paper with glue †.

Analyte	DBS-No Glue	Glue Undiluted	Glue Diluted (1:1)	Glue Diluted (1:4)
OHP	0.35 ± 0.21 (60.6%)	0.78 ± 0.04 (4.6%) *221%*	0.48 ± 0.32 (67%) *136%*	0.73 ± 0.32 (43.9%) *207%*
T4	42.9 ± 1.84 (4.3%)	47.3 ± 6.15 (13%) *110%*	37.1 ± 1.77 (4.8%) *86%*	35.6 ± 2.47 (7%) *83%*
TBG	156 ± 1.41 (0.91%)	195 ± 12.4 (6.3%) *125%*	183 ± 22.3 (12.2%) *117%*	145 ± 17.7 (12.2%) *93%*
BIOT	85.5 ± 7.78 (9.1%)	109 ± 23.3 (21.5%) *127%*	110 ± 4.24 (3.9%) *129%*	96 ± 19.8 (20.6%) *112%*
GALT	16.2 ± 0.99 (6.1%)	19.8 ± 3.11 (15.7%) *122%*	17.6 ± 1.98 (11.2%) *109%*	16.3 ± 0.28 (1.7%) *101%*
IRT	22.5 ± 0.71 (3.1%)	25.0 ± 0 (0%) *111%*	20.5 ± 0.71 (3.4%) *91%*	22.5 ± 0.71 (3.1%) *100%*
PAP	2.58 ± 0.02 (0.91%)	2.95 ± 0.16 (5.6%) *103%*	3.02 ± 0.02 (0.8%) *98%*	2.70 ± 0.38 (14.0%) *99%*
Leu	83.6 ± 3.49 (4.2%)	87.5 ± 1.51 (1.7%) *105%*	95.5 ± 1.14 (1.2%) *114%*	87.8 ± 6.11 (7%) *105%*
Phe	32.6 ± 1.37 (4.2%)	35.1 ± 0.01 (0.02%) *108%*	37.3 ± 0.06 (0.2%) *115%*	35.0 ± 1.48 (4.2%) *107%*
Tyr	31.7 ± 0.76 (2.4%)	33.5 ± 3.54 (10.6%) *106%*	35.8 ± 4.07 (11.4%) *113%*	32.9 ± 3.3 (10%) *104%*
Val	96.6 ± 3.54 (3.7%)	99.4 ± 1.68 (1.7%) *103%*	112 ± 6.83 (6.1%) *116%*	101 ± 5.51 (5.4%) *105%*
C0	19.2 ± 0.62 (3.2%)	19.5 ± 1.22 (6.3%) *101%*	21.8 ± 0.36 (1.7%) *113%*	19.7 ± 1.05 (5.3%) *103%*
C5-OH	0.41 ± 0.04 (10.4%)	0.32 ± 0 (0%) *78%*	0.47 ± 0 (0%) *115%*	0.47 ± 0.08 (16.7%) *113%*
C8	0.11 ± 0 (0%)	0.11 ± 0.02 (20.2%) *95%*	0.12 ± 0.01 (6.1%) *105%*	0.1 ± 0.01 (14.1%) *91%*
C14:1	0.06 ± 0.01 (12.9%)	0.06 ± 0.01 (12.9%) *100%*	0.06 ± 0.01 (12.9%) *100%*	0.04 ± 0.01 (20.2%) *64%*
C16:1-OH	0.01 ± 0 (0%)	0.01 ± 0 (0%) *100%*	0.01 ± 0 (0%) *100%*	0.01 ± 0 (0%) *100%*

Note 1: Data presented is mean concentrations of duplicate measurements ± 1 SD (%CV). Note 2: Presented results are from analytes for which measured concentrations were in the quantifiable range only. † Units in blood: OHP, T4, TBG, nmol/L; BIOT, % c.f. daily mean; GALT, U/dL; IRT, μg/L; Leu, Phe, Tyr, Val, C0, C5-OH, C8, C14:1, C16:1-OH, μmol/L. Concentrations are also expressed as percentage of concentrations in the absence of glue (second line, italics).

4. Discussion

We have conducted an investigation into the effect of glue, used in the manufacture of Whatman#903 NBS cards. This study has evaluated the effects on analytes measured as routine in the Dutch NBS programme. Adult whole blood was used in this study without enrichment: for all markers except OHP, analyte concentrations were in the quantifiable range, and for the majority of markers, analyte concentrations were comparable to average concentrations detected in neonate samples analyzed in our laboratory. Data from selected analytes, for which measured concentrations were in the quantifiable range and which reflect the range of analytes and analytical methods, illustrate our

findings. In these experiments, we simulated a situation where a considerable glue spill directly contaminates the filter paper. Contamination of the filter paper with glue during the manufacturing process is highly unlikely, especially at glue concentrations assessed in this work. These data indicate that the presence of glue, at three concentrations at the upper limits of the risk spectrum, has no effect on the measured concentrations of analytes employed in the Dutch NBS programme.

A logarithmic relationship between spot volume and spot diameter has previously been described, and a DBS diameter of 11.5 mm was reported for a spot volume of 50 µL [4]. Our results for DBSs on Whatman#903 concur with these data. However, our results show that the diameter of the DBSs prepared in the presence of glue, undiluted and diluted, is approximately 20% larger than the DBSs prepared in the absence of glue. An increase in spot diameter might be expected to reduce analyte concentration, but in this study a corresponding, structural decrease in measured analyte concentrations was not observed in the presence of undiluted glue.

DBSs prepared in the presence of undiluted glue were uniform in shape and blood distribution. However, when the glue is diluted with demineralized water (1:1 and 1:4, glue/demineralized water), the DBS is non-uniform with respect to shape and distribution of the blood on the filter paper. The CLSI Approved Standard NBS01-A6 [5] states that the acceptable range for absorption of 100 µL blood is 5–30 s. If a linear relationship between blood volume and absorption time is assumed, then for 50 µL an acceptable range for absorption time is 2.5–15 s. Our results for DBSs produced under all conditions lie within these limits. DBSs produced in the absence of glue or in the presence of undiluted glue are similar (4.06 s and 4.23 s, respectively). However, absorption time for DBSs produced in the presence of diluted glue are markedly higher (80–90%). In addition, once absorbed, the distribution of the glue on the filter paper was uneven. From these observations, it can be concluded that the property of the glue and the manner in which the glue interacts with the filter paper is altered following the addition of demineralized water. Upon mixture, the glue appeared to be miscible with demineralized water; however, upon standing, two phases formed. It is possible that, because of the addition of demineralized water to the glue, its physical properties change such that hydrophilic and hydrophobic areas form when the glue is applied to the filter paper. A definitive conclusion for this effect cannot be elucidated from this study and is beyond its scope. The study design included the dilution of the glue with water to create different glue concentrations in order to determine if any effect of glue contamination was proportional to the concentration of glue. However, since glue contamination at the highest concentration (undiluted glue) had no effect on the measured concentration of analytes, the proportional effects of different glue concentrations were of less relevance.

Interference has been assessed by comparison of results obtained from DBSs prepared in the presence and absence of glue. No interference was identified for the analytes, analytical methods, and instruments included in this study. The measured concentrations of OHP were close to the limit of quantification (0.1 nmol/L) and were significantly higher in blood prepared in the presence of glue (at all glue concentrations). This effect was not observed for analytes measured using similar methods or instrumentation (T4, GALT, IRT), indicating that it is not the analytical method or analytical instrument that is sensitive to the presence of glue. Adult blood was used to prepare the DBSs and since OHP levels in blood from a healthy adult are typically less than half of the levels found in a healthy neonate, it can be concluded that the low levels of OHP in the blood and the corresponding high variability in the raw data have incidentally given rise to this effect.

5. Conclusions

In the process of producing heel prick cards using Whatman#903 and the EBF#1003 glue, contamination of the filter paper with EBF#1003 glue is extremely unlikely. Contamination of filter paper with undiluted glue should be apparent from a visual check of the card, but will not affect the size or shape of a DBS. The data from this study show that the glue will not affect the results of NBS analysis; therefore, contamination is unlikely to be responsible for unexplained deviant measurements in routine neonatal screening.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Adam, B.W.; Alexander, J.R.; Smith, S.J.; Chace, D.H.; Loeber, J.G.; Elvers, L.H.; Hannon, W.H. Recoveries of phenylalanine from two sets of dried-blood-spot reference materials: Prediction from hematocrit, spot volume, and paper matrix. *Clin. Chem.* **2000**, *46*, 126–128. [PubMed]
2. Mei, J.V.; Alexander, J.R.; Adam, B.W.; Hannon, W.H. Use of filter paper for the collection and analysis of human whole blood specimens. *J. Nutr.* **2001**, *131*, 1631S–1636S. [PubMed]
3. De Jesus, V.R.; Mei, J.V.; Bell, C.J.; Hannon, W.H. Improving and assuring newborn screening laboratory quality worldwide: 30-Year experience at the centers for disease control and prevention. *Semin. Perinatol.* **2010**, *34*, 125–133. [CrossRef] [PubMed]
4. *Newborn Screening Quality Assurance Program*; Filter Paper Comparison Study; Centers for Disease Control Prevention (CDC) and the Association of Public Health Laboratories (APHL): Atlanta, GA, USA, 2009; pp. 30341–33724. Available online: https://www.cdc.gov/labstandards/pdf/nsqap/nsqap_FilterPaperStudy51809.pdf (accessed on 12 November 2015).
5. Clinical and Laboratory Standards Institute (CLSI). *Blood Collection on Filter Paper for Newborn Screening Programs*, 6th ed.; CLSI Document NBS01-A6; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2013.
6. Hall, E.M.; Flores, S.R.; de Jesus, V.R. Influence of hematocrit and total-spot volume on performance Characteristics of Dried Blood Spots for Newborn Screening. *Int. J. Neonatal Screen.* **2015**, *1*, 69–78. [CrossRef] [PubMed]