National Newborn Bloodspot Screening Programme

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Blood spot quality – how this affects results and common errors with sample collection and recording information

27.3.25

Effectiveness of Screening

Screening is only effective if it can reliably detect babies with a particular disease before the baby becomes symptomatic. Effectiveness can be assessed by the number of false positives and negatives.

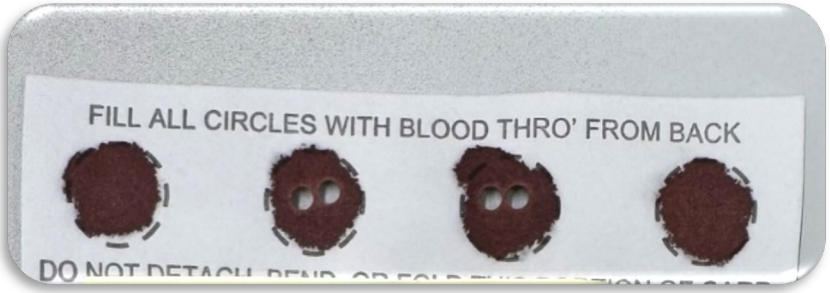
- **False positive** (FP) happens when a newborn does not have the disease, but the results are incorrectly positive, the newborn undergoes follow-up diagnostic testing which can be invasive and cause parental anxiety.

- **False negative** (FN) can have much more catastrophic consequences and can occur when the screening result incorrectly determines the baby does not have the disease. The baby will not receive presymptomatic treatment and so the disease symptoms will manifest.

Pre-analytical variation due to poorly collected samples is a source of imprecision and can result in a FP or FN screen result if sample quality is not adequate.

Dried Blood Spot Sample Quality

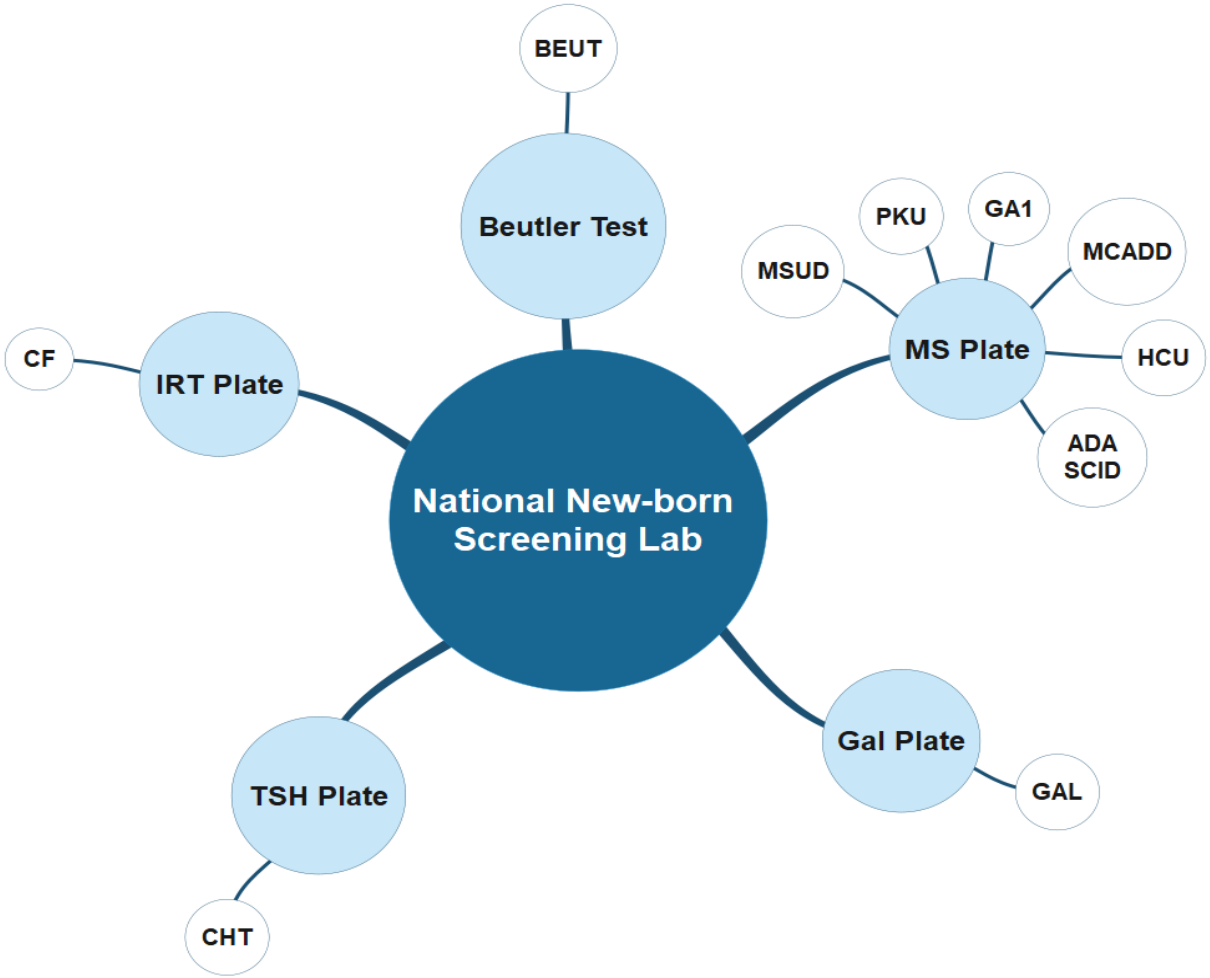
- There are several quality issues that can affect screening results, such as sample contamination, formation of serum rings or if a sample have been compressed.
- However, the most significant is the volume of the blood contained within a 3.2mm punch which is required for accurate testing and can have a significant impact on the reliability of measurements taken from the spot.
- The smaller the spot the more reduced the result.
- The spreading of blood components, plasma and erythrocytes is not uniform and makes the volume of blood in the 3.2mm punch inaccurate.
- 10 x 10 mm is the ideal good quality spot.



Punched plates with samples in test wells

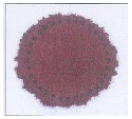
NNBSL Workflow - different processes for each disorder

SMA & SCID molecular testing – validation due to start Q2 2025



The NBS card is a medical device

- The filter paper used to manufacture NBS cards is classed as a medical device, is CE marked and meets international Clinical and Laboratory Standards Institute (CLSI) standards.
- Particle retention, pore size and thickness all determine the volume of blood the paper can absorb, this is known as the loading capacity and the spreadability of the blood.
- The method employed to apply the blood to the filter paper can play a role in the kinetics of blood spreading. That is, if the 'Hanging Droplet' technique is used and blood is allowed to form properly and drop onto the filter paper, as opposed to touching or rubbing the filter paper to the heel.



Concentrations of all compounds measured are higher in the periphery of a blood spot, but this is more pronounced for analytes that are erythrocyte associated, such as TSH (CHT), IRT (CF) and Methionine (HCU) which are either solely present in plasma or in higher concentrations, and therefore if the spot is small in diameter analytes concentration will be less.

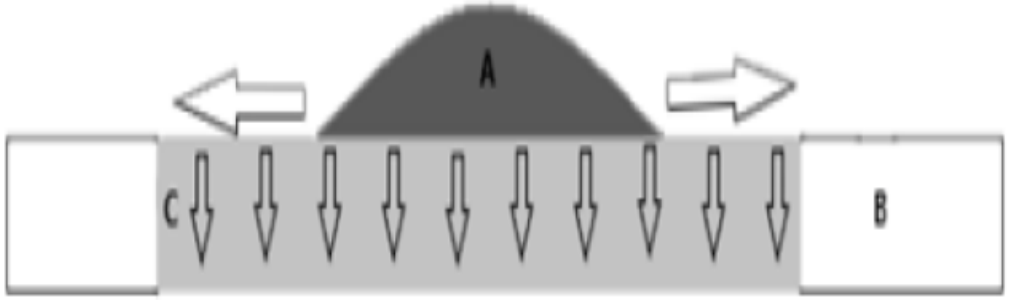
The hanging droplet technique

The gold standard method

- The capillary blood is allowed to flow from the puncture site until a large droplet forms on the heel. The droplet is suspended from the heel until it reaches critical volume capacity and then it drops onto the filter paper.
- The blood should be dropped into the centre of the pre-printed 10mm circles, to allow radial dispersion to the edges of the circle.
- The blood, when applied in this manner, rapidly spreads radially across the filter paper whilst simultaneously fully soaking the paper by penetrating the porous fibres.
- It is the effect of having the ‘hanging droplet’ drop onto the filter paper that allows uniform spreading to happen.

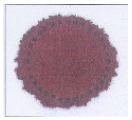
Blood Application & Kinetics of Blood Spreading

Kinetics of Blood Flow Through Filter Paper



Note. A – blood droplet, B – filter paper, C – area of spread (wetting area). The arrows indicate the flow of blood simultaneously through and across the filter paper

- A single hanging drop of capillary blood of adequate size should be sufficient to fill the pre-printed 10 mm circle on the screening card. Over or under filling the pre-printed circle affects the volume of blood in the punch.



10 mm spot

- When blood is applied in other ways, the spreading through the paper matrix will not happen in the same way, and is less likely to be uniform, e.g. heel tapping.

- **Heel tapping** - As the blood flows from the puncture site and forms as a droplet on the heel, if the blood is collected on the filter paper by tapping the paper to the heel, the blood applied will be less because critical ‘dropping capacity’ has not been able to be achieved.

Drying/transport - Adequate drying is essential as this stabilises analytes, thus allowing longer time periods between taking the sample and analysis, without compromising results. This is why NBS is possible when performed in the community as opposed to venous samples for other biochemical blood workups.

- Stability of the analytes and enzymes can be affected by rapid drying or poorly dried samples, some analytes can decrease in concentration by 20% to 40% with a risk of a false negative result.
- If the sample is not dry when it is put in an envelope, blood will leach from the filter paper onto the envelope, altering the blood volume. The spreading of blood components plasma and erythrocytes will not be uniform making the volume of blood held in a 3.2mm punch inaccurate.
- Samples should arrive in the laboratory within one-two days, at ambient temperature not be exposed to heat or humidity.

The effect of sample volume means that anything that alters the volume of blood in a 3.2mm punch needs to be considered as a sample quality issue, this includes overall blood spot size, application technique such as layering or multi-spotting, and compression of the sample.

For this reason

- All samples identified as having an issue that could affect blood volume must have a repeat sample taken.
- Unfortunately determining sample quality is not straightforward as variations within the sample are not always visible.
- The quality of all samples received into the laboratory are assessed visually to ensure that; volume is sufficient, the blood is spread evenly on both sides of the filter paper and that the following poor quality issues are not present.

Poor quality samples which occur by poor application technique can be categorised as follows

- **Insufficient** - small sample, where bloodspot is less than the recommended 10 mm.
- **Blood not soaked through** – sample not taken using the droplet technique and blood did not permeate through, leading to an insufficient volume collected.
- **Layered sample** – spotting blood over blood to fill a circle likely due to the first drop being insufficient, a barrier can form and the second droplet is prevented from penetration and therefore a fully saturated spot is not achieved.
- **Multi-spotting** - is when several small spots are applied to the paper which may all join together to create one big spot. There will be patches of overlapping, and patches where there is only a single layer of blood. Multi-spotting will significantly decrease the homogeneity across the spot.

- **Compressed samples** - occur when the sample is ‘squashed’ or pressed down. The squashing process forces the blood out from the air pockets in the filter paper and through the non-soaked parts of the filter paper. The result is that a small volume of blood will spread through and across the card much further, but will be diluted in the centre and may give a false negative result.

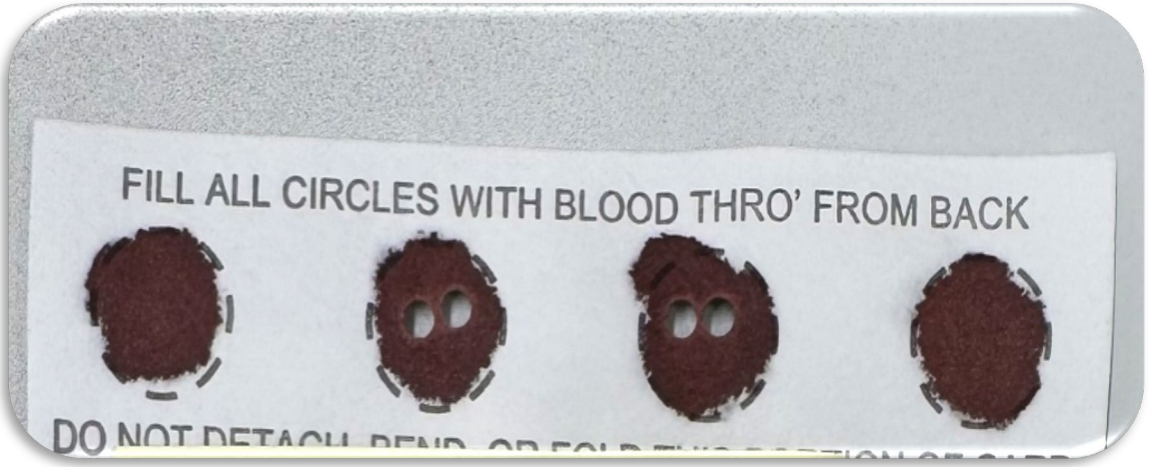
- **Wet samples -** run the risk of compression, causing the collapse of the microscopic pores between the filter paper, thus reducing the volume the filter paper can hold. These samples will inherently contain lower volumes of blood than non-compressed samples but will visually look very similar and may produce a false negative result, in some cases causing analyte levels to drop by up to 40%.

- **Blood applied to both sides of the filter paper** - If applied to the front and back of the card the size and shape on each side of the card is different and there are areas where the samples do not meet, and therefore the blood volume is reduced. Alternately, the volume of blood added to each side increases the overall volume as some blood sits on top of the filter paper as it is not able to be fully absorbed into the filter paper.

Preferably four, fully filled circles (10 mm diameter), evenly saturated with a single drop of blood per circle is the ideal sample. This allows for routine screen plus the possibility that a sample may need to be repeated due to instrument or QC failure in the laboratory process, or if abnormal results and duplicate analysis is required to check results.

- Blood spots must be allowed to dry sufficiently before being placed in an envelope.
- Samples must NOT be packaged while wet.

- Picture of good quality cards received



Other considerations for rejection of samples not relating to bloodspot quality include;

- **Delay in receipt of sample:** samples received >14 days post collection are rejected as there will have been deterioration of analyte level and potential false negative results, e.g. HCU screen deteriorates quickly. Consequently there could be a delay in Dx and treatment.

Relevant Family Hx/Meconium Ileus

Meconium Ileus is a common complication in babies with CF, occurring in approximately 18% of all babies with CF in Ireland. Not all these infants present with a raised blood IRT (i.e. positive CF screen) from the newborn screen. Therefore, CF should be considered in all babies who present with meconium ileus within the first few days of life.

Information about meconium is not required, only meconium ileus.

NB.; We do not need to know non-NBS info, e.g. mum had depression etc

Recording correct LHO or HSE Local Health Area and why?

- It is the responsibility of the sample taker to ensure that the correct area that results are to be returned to is noted.
- If not completed the lab sends the report to the relevant area after using the ***'HSE Atlas address finder tool'*** **or** based on address provided, e.g. address is Co. Kilkenny then results go to CC Kilkenny.
- However, the lab is unable to continuously search for these missing LHOs when not assigned and each extra step delays the process for admin staff.

The ***HSE address finder*** is a valuable tool to assign correct LHOs & should be used by sample takers if unsure of LHO.

Where do results go to, how long are they held, how can I look them up?

All results go to both community care head office and hospital (normal & abnormal), and are available electronically on eReports for 60 days (*archived from eReports after that due to storage limitations on server*).

It is the responsibility of each head office to issue results to their PHNs and requests for results should therefore go through respective CC head office and not laboratory. We received many call for results that have been reported.

Poor legibility and incorrect completion of NBS cards is a recurring issue
Cards should be sent with an addressograph label preferably; a considerable workload is created for all of us amending patient details.

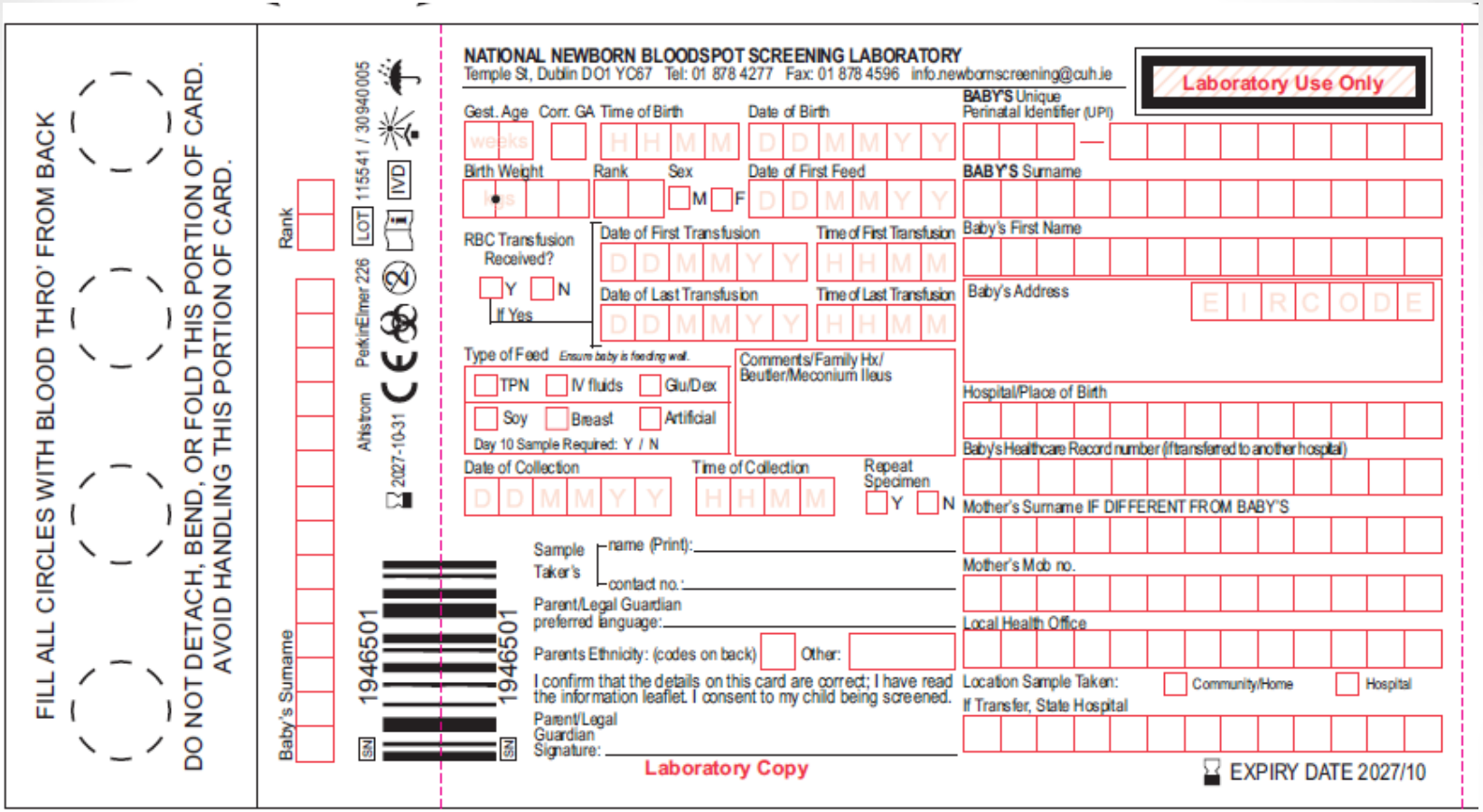
An outline of steps involved in investigating and correcting errors:

Wrong or illegible details noted - Result sent to incorrect area - Result cannot be found on eReports due to being incorrect– PHN phones head office/lab querying status of result - Issue investigated - Confirmation required in writing- Lab admin staff correct details-Scientific staff then review, edit and amend report accordingly -Correct report the issued.

All above can take 1+ hrs. of all our time, we get a large number each week 😲

Disclaimer: Some of these errors are lab created and we monitor as a KPI and try improve internally.

Requesting Copy Results - First request by PHNs should be to their own head office, do not use NNBSL as default for getting reports!
If head office can't locate results on eReports for whatever reason they then contact NNBSL.
Why cant we find reports: was wrong LHO/Address/ Demographics of baby recorded on card/Illegible details?



Data requested on NBS card includes:

- **Feeds type:** if Breast/Artificial/IV/ Glu/Dex/TPN or Soya
- **Reminder to ensure adequate feed intake & prompt repeat card**
- **RBC Transfusion status, Gestational age, Weight, Ethnicity & Signed consent**

There is a reason why we require all this information, to identify a baby and for result interpretation

MONTANA DPHHS

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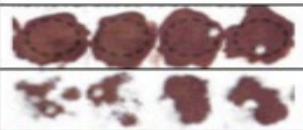
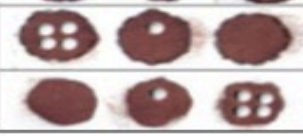
Steve Bullock, Governor

Sheila Hogan, Director

MONTANA NEWBORN SCREENING: BLOODSPOT SPECIMEN QUALITY CHECK

Problem	Examples	Causes	Prevention
Good Spots (front & back)			
Insufficient quantity: Circles not filled with blood		Collector unable to obtain large drops of blood from heel.	Hydrate the baby; warm and lower the heel; puncture again.
Insufficient quantity: Filter paper not saturated (front and back of same card)		Blood applied to each circle did not soak through evenly.	Apply one large drop per circle; check reverse for soak through; don't touch sample area.
Filter paper damage: Creases and tears		Wet filter paper is easily damaged.	Do not overload card or touch the wet sample; do not crease.
Filter paper damage: Capillary abrasion		Capillary scraped on wet filter paper.	Avoid capillary tubes if possible; never touch capillary to card.
Poor quality: Layered specimen		Collector unable to apply large drops of blood.	Apply one large drop per circle; never add to a partially dry spot.
Poor quality: Contamination		Blood contaminated by liquid absorbed on card after blood applied.	Dry the cards flat away from spilled liquids.
Poor quality: Serum rings		Serum or tissue fluid separates from blood cells on card.	Dry flat; apply gentle heel pressure rather than "milking".
Poor quality: Clotted specimen		Delayed application of blood to card using capillary or syringe.	Avoid devices; if used, need one per spot ; no anticoagulant.
Poor quality: Blood applied to both sides (smearing front and back of same card)		Smeared blood on both sides suggests blood applied to both.	Apply blood to one side only. Dry flat for at least 4 hours before closing flap.

If you see an unsatisfactory specimen, please collect another right away.

The delay caused by an unsatisfactory sample could be life-threatening to an affected child!

www.newborn.hhs.mt.gov

References on bloodspot quality

1. Guidelines for Newborn Blood Spot Sampling (Public Health England). https://www.gov.uk/government/publications/newborn-blood-spot-screening-sampling-guidelines
2. Moat SJ, George RS, Carling RS. Use of Dried Blood Spot Specimens to Monitor Patients with Inherited Metabolic Disorders. International Journal of Neonatal Screening. 2020; 6(2):26. https://doi.org/10.3390/ijns6020026
3. Guidance - Newborn blood spot screening: laboratory guide for IMDs (Public Health England). https://www.gov.uk/government/publications/newborn-blood-spot-screening-laboratory-guide-for-imds
4. CLSI. Dried blood Spot Specimen Collection for Newborn Screening. 7th edition. CLSI standard NBS01. Clinical and Laboratory Standards Institute; 2021.
5. George, R.S. and Moat, S.J. Effect of Dried Blood Spot Quality on Newborn Screening Analyte Concentrations and Recommendations for Minimum Acceptance Criteria for Sample Analysis. Clinical Chemistry 2015; 62(3): 466–475.
6. Moat SJ, Dibden C, Tetlow L, Griffith C, Chilcott, Hamilton L, Wu THY, MacKenzie F, Hall SK. Effect of blood volume on analytical bias in dried blood spots prepared for newborn screening external quality assurance. Bioanalysis 2020, 12(2): 99-109.

THANK YOU

The majority of samples are collected correctly and have details completed accurately, but for the ~3-5% that don't, it results in re-bleeding of newborns, extra work for all involved and delays in issuing results.

Also referred to a Avoidable Repeats

Thanks to all PHNs, Midwives and NNBS Laboratory staff for their continued support of the programme, dedication and hard work over the years as we progress with expanding the programme to include SMA & SCID screening and moving the service to the New Paediatric Hospital in St James site, Dublin which is a major challenge when continuing BAU.

Our new workplace, date tbc

- of note laboratories are all at level - 01, best rooms are for patients with views of Dublin city and mountains