Meeting Report

ISNS 9th International Symposium, The Hague, The Netherlands, September 11–14, 2016

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1. Introduction

The International Society for Neonatal Screening (ISNS) was established almost 30 years ago by a small group of international pioneers in the newborn screening field. They decided to go public in Leura, NSW, Australia, in 1991 with the first ISNS International Symposium. This year we celebrate their great efforts leading to that event of 25 years ago with a silver jubilee symposium.

Because of this milestone the local organising committee wishes to highlight a World Health Organisation book which was prepared nearly 50 years ago. It was entitled "Principles and practice of screening for disease" and is often referred to as the "Wilson and Jungner Criteria". The opening session on Sunday, September 11 will focus on the background to the book and its authors. In other sessions the application of the Wilson and Jungner Criteria in daily practice are highlighted.

The rest of the programme will offer you a range of interesting topics, varying from "what is being screened where", via new technological developments for conditions that have been screened for many years, and new conditions that are not yet commonly screened, to the newest challenges offered by the potential introduction of genotypic screening. There will be two lunch sessions focussed on Critical Congenital Heart Disease and Dried Blood Spot Analytical Phenomena, respectively.

Below you will find the abstracts grouped as invited presentations, oral presentations and poster presentations.

2. Invited Presentations

I01. Biological Standards for Diagnostics—Development, Value Assignment and Commutability

Paul Stickings

National Institute for Biological Standards and Control, UK

NIBSC is a world leader in the development, production and distribution of biological standards covering a wide range of product types from nucleic acid material to purified proteins and whole bacteria. These standards are typically calibrated in units of biological activity and their use enables consistency in measurements of biological activity. NIBSC develops standards for diagnostic use that cover a range of infectious disease markers, diseases of blood and endocrine systems and genetic disorders. Appropriately calibrated diagnostic standards are essential for standardisation of measurements from different laboratories and different assay systems. Because most diagnostic measurements for biological analytes are made independently of a reference method, it is important that any higher order reference material (to which measurements are ultimately traced) is commutable for the methods that are in common use for the analyte in question. This presentation will give an

overview of the development of diagnostic standards for biological analytes with a focus on value assignment and commutability.

I02. The Preparation and Use of CDC's Newborn Screening Quality Control Material

Carla Cuthbert

CDC, Atlanta, GA, USA

The Newborn Screening and Molecular Biology Branch, at the Centers for Disease Control and Prevention (CDC), operates the Newborn Screening Quality Assurance Program (NSQAP). NSQAP is a voluntary, non-regulatory program that helps to assure early and accurate laboratory detection of congenital disorders in newborns.

The program prepares and distributes more than 700,000 dried blood spot (DBS) quality assurance materials per year to both U.S. and international laboratory participants.

The DBS materials manufactured at CDC are designed to simulate, as closely as possible, the actual patient specimens tested and are assessed for homogeneity, accuracy, stability, and suitability for use within newborn screening programs.

CDC's quality control materials are distributed twice per year and program participants are expected to return quantitative results from five analytical runs of these materials. These quality control materials are intended be used as external quality controls to assist in maintaining continuity of testing evaluation and transcend changes in production lots of routinely used method- or kit-control materials. They can be used for monitoring of the long-term stability of assays and are not meant to be used as routine daily quality control materials. Of note, CDC's quality control materials should not be considered to be calibrators or reference materials.

Through interactive efforts with its program participants, CDC continues to strive for improvements in services offered and to meet the growing and changing needs for newborn screening in the public health community.

I03. ISNS Reference Preparation for Newborn Screening (RPNS) for Thyroid Stimulating Hormone, Phenylalanine and 17-Hydroxyprogesterone

Veronica Wiley [1], Bert Elvers [2] and Gerard Loeber [2]

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Background: The International Society for Neonatal Screening (ISNS) has prepared reference material in dried blood spot samples for use by kit manufacturers and QA programme providers on 4 occasions since 2004. The current preparation is due to expire in December 2016.

Aim: To determine the consistency between the ISNS Reference Preparations considering all aspects of measurement of uncertainty.

Method: The method for preparation of each RPNS was based on that published for the 1st preparation (Elvers et al. *JIMD* **2007**, *30*, 609). In brief, blood was collected from a healthy volunteer; the basal blood was tested for haematocrit and then divided into 6 aliquots. Stock solutions of thyroid stimulating hormone (TSH), phenylalanine (Phe) and 17-hydroxy progesterone (17OHP) were prepared. Each blood portion had differing volumes of stock solutions, saline and red cells added so that the final volume of each vial was the same with a haematocrit of 50% $\pm$ 0.5%. The spiked blood was spotted onto filter paper cards, allowed to dry overnight and then stored at $-20\,^{\circ}$C with desiccant. The 6 different concentrations of each analyte in dried blood spot format were calculated from the basal concentration measured in plasma and blood and the weighed amounts of analyte added to the blood. The final concentration was checked for homogeneity at various laboratories.

Results: The determined recovery for each analyte in each of the 4 preparations was on average 95% to 100% with CVs of approximately 10%. The average differences between each of the 4 preparations was <10%.

Conclusions: The use of a reference preparation by kit manufacturers and QA programmes provides harmonisation of quantitation for TSH, Phe and 17OHP internationally. This therefore allows comparison of quantitative results and determination of action limits to be consistent between newborn screening programmes.

I04. Wilson and Jungner's Criteria, Are They Still Useful? A Public Health Perspective

Angela E Raffle

UK National Screening Programmes, Bristol, UK

My first involvement in screening was in 1983 with Anne Green helping set up a register of all PKU children in the English West Midlands. I then became deeply involved in cervical screening, publishing data from my own local programme that made it hard for people to keep ignoring the major problem of overdiagnosis and overtreatment in cancer screening. I was part of the network of people who created the UK National Screening Programmes. I was aware of how the Wilson and Jungner Criteria had been misinterpreted and misused over the years. I was aware also of the different perspectives of clinicians—who see the one case that could have been prevented, versus the perspective of public health specialists—who see the whole population and all the potential harms. Our aim in the UK National Screening Programmes is to make evidence-based policy decisions, at national level, to ensure we introduce only screening that will do more good than harm at affordable cost. Our aim is to ensure nationwide quality assured programmes that deliver maximum benefit and minimum harm. Criteria are difficult because they reduce complex and nuanced questions to a set of apparent dichotomies—yes/no, black/white. Yet criteria are essential if we are to have a legally robust process. The UK NSC criteria are reviewed regularly and probably are about as good as they can be. My experience tells me that our best work happens when different professions listen carefully to each other, and when we keep the best interests of the public we serve at the forefront of what we do.

I05. Wilson and Junger—An Ethical Perspective

Wybo Dondorp

Maastricht University, Maasticht, The Netherlands

Wilson and Jungner (WJ) wrote their landmark report for the WHO not only on the eve of a whole new era of genetic and reproductive screening, but also prior to important cultural changes that frame the normative context for current debates about screening policies. Firstly, whereas in WJ the Hippocratic principles of beneficence and harm avoidance form the core of their ethics, current perspectives turn on balancing these principles with an emphasis on autonomy. Secondly, whereas in WJ all population screening has the 'simple' aim of improving health outcomes for the population, additional aims that have since been formulated include the provision of reproductive choices or personal utility. Instead of regarding these developments as showing that we have moved beyond WJ, it would be more appropriate to say that their legacy is the dynamic framework for continuing debate about how under new circumstances, population screening can do more good than harm. More than any other screening context, newborn screening is a battle ground for different approaches to this debate. This is because in no other screening context there is so much moral complexity. This complexity will only grow larger with the introduction of genomic newborn screening, for instance with regard to dealing with later onset findings. When moving into this new territory, we continue to need WJ as an important ethical beacon, reminding us that any offer of screening can only be responsible if the balance of benefits and harms for those tested is clearly positive.

I06. Current Status of Newborn Screening in North America

Bradford Therrell

U.S. National NBS and Global Resource Center, Univ of TX Health Science Center at San Antonio, Austin, TX, USA

The status of newborn screening in North America was previously published in 2007 and updated in 2015 along with information from other regions of the world. Currently in the U.S. there continues to be no national newborn screening program and the same is true in Canada. Following recommendations from a special workgroup of the American College of Medical Genetics and Genomics, and their acceptance by the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children in 2005, the U.S. Secretary of Health and Human Services formally endorsed a Recommended Uniform Screening Panel (RUSP) of 29 core conditions and 25 secondary targets (conditions not meeting the criteria for "core" status but possibly revealed during screening or differential diagnosis of a "core condition") as a means of encouraging national screening panel harmonization. Since the original recommendations in 2005, which were based on an empirical point scoring system, a more formal nomination and evidence review process has been implemented. A number of conditions have been nominated for consideration for addition to the RUSP but only a few have been accepted. Currently there are 34 conditions on the RUSP and 26 secondary targets. This presentation will briefly review the U.S. national recommendation process and provide an update on the conditions currently recommended at the national level and their implementation progress. Similar data will be presented for the Canadian Provinces.

I07. Current Status of Newborn Screening in Latin America

Gustavo Borrajo

Detección de Errores Congénitos. Fundación Bioquímica Argentina. La Plata, Argentina

Newborn Screening (NBS) in Latin America began its first organized activities three decades ago when the Cuban national NBS program was implemented. From then onwards, it experienced a slow but steady growth, currently showing a continuous and complex spectrum of situations. Cuba (1986), Costa Rica (1990), Chile (1992) and Uruguay (1994) have the longest experience in the implementation of national programs with optimal fulfillment. Brazil (2001), Argentina (1995 and 2006) and Mexico are in a second level, and although they have showed significant improvements in coverage and diseases screened, they need to work in order to strengthen objectives beyond the coverage. Colombia (2000) and Venezuela (1999) have a long NBS history, however they screen systematically only for one or two classical diseases, having pilot projects for NBS expansion in the coming years. Paraguay (2003), Panama (2007) and Nicaragua (2005) have experienced a progressive coverage increase and an expansion in their panels since the NBS implementation except Nicaragua, where only Congenital Hypothyroidism is screened. Ecuador (2011), Peru (2012) and Bolivia are the more recent countries implementing NBS programs, but they are yet dealing with difficulties own of such early organization phase. Guatemala, Dominican Republic and El Salvador have not exhibited significant NBS changes in the last years, while Honduras and Haiti have not any known activity. Congenital Hypothyroidism is the most widely screened disease, existing organized programs running in 16 countries. NBS by MS/MS is conducted systematically only in Costa Rica and Uruguay, being also offered as pilot or from private sector in other seven countries. Every year, approximately 11 million infants are born in Latin America, having access to NBS benefits around 68% of them.

I08. Current Status of Newborn Screening in Europe

J. Gerard Loeber

ISNS Office, Bilthoven, The Netherlands

Over the last 50 years almost all European countries have introduced neonatal screening as an important public health feature. Depending on health care structure, available funds, local politics, input from professional groups and the general public, this introduction has led to different approaches in the way the screening programmes have been set up, financed and governed. To get more information on these differences, in 2010 an online survey, commissioned by the EU, was compiled in which the whole screening programme was covered by a questionnaire. This survey covered the EU member states, (potential) candidate member states and EFTA countries, in total 38 countries. Results showed large variations in the panel of screened conditions, ranging from 0 to more than 30 conditions; specimen collection time after birth; screening methodology; and storage of residual specimens, varying from 1–1000 years. In addition, confirmatory diagnostics, treatment, and follow-up showed large discrepancies. In 2011 the project group provided a list of 60 recommendations to the EU Commission, but so far none of them have been taken up.

Recently the same colleagues were asked to update their data. Over the last five years, in some, mostly smaller, countries considerable changes have been implemented, mainly concerning the number of ms/ms detectable conditions. In contrast, in other mainly larger, countries very little has changed, if at all. Screening for SCID and CCHD, very much promoted and widely implemented in the USA, is getting attention but so far only in pilot programmes in a few countries. In contrast to the US, in Europe national public health policies are either not at all, or only marginally, influenced by developments in neighbouring countries. It is therefore unlikely that NBS programmes in Europe will converge in the years to come.

I09. *Current Status of Newborn Screening in Middle East-North Africa*

Issam Khneisser

Saint Joseph University, Newborn Screening Laboratory, Beirut, Lebanon

Newborn screening coverage is soaring in the MENA region since the ISNS initiated meetings 2006, 2008 and 2010 even with man-made calamities since the Arab spring in 2011.

Many countries reached 99% coverage of at least two diseases United Arab Emirates, Egypt, Jordan, Kuwait, Palestine; other have 100% coverage with expand panel like Qatar and major region in Saudi Arabia. Iran had also a national program for CH screening supported by IAEA.

Bahrain has their local program sickle disease and shipping abroad, to Saudi Arabia mainly, for screening support with a high a coverage around 70 percent. The government took a decision for a national newborn screening program.

Lebanon has 2/3 of newborns benefit from an expanded newborn screening panel. The government is only providing the follow up. The screening is done on private efforts.

Oman had no national program for the time being. They had regional and international support during the last two decades and ready to start. Efforts like recording a sung "video clip" addressed to the prince of the Sultanate Oman by all treated Inborn of Metabolism patients.

Morocco supported closely by JICA, they have a platform for CH screening in Rabat center with 20.000/year. Casablanca center acquired the platform in mid-October and a new platform will be installed in FES.

Iraq did acquire platforms in many districts mainly Baghdad, Arbil. CH screening started but coverage is still around 25%.

Algeria supported by many members of ISNS since mid-1990s. Still nothing is done, only short lived pilot programs. A tremendous work had been done lately; they did establish a "road map"; for a national program supported by UNICEF. A green light for national program is waiting at the Minister's office, the same issue is in Tunisia. ISNS endorses some efforts in these two countries in the coming two years to push towards the start-up.

Libya, Yemen, Syria had many screening pilot programs. With their current man made calamities, a chaotic status currently taking place.

I10. *Newborn Screening Program Developments in the Asia Pacific Region*

Carmencita Padilla

University of the Philippines Manila, Newborn Screening Reference Center -National Institutes of Health, Ermita, Metro Manila, Philippines

The Asia Pacific Region extends from New Zealand on the South to Mongolia on the North, and reaches to Pakistan in the East. Of the 138 million babies born in the world, almost half (67 million) are born in the Asia Pacific Region. Countries in the region vary widely in size, economic development and geography. There are many different languages, cultural sensitivities, and religions, each creating its own challenges in implementing NBS. There are currently more developing programs than developed programs within the region. Despite these challenges, NBS continues to grow throughout the region. Infant mortality rate (IMR) has been found to be a good predictor of when competing health issues acknowledge the need for NBS, and all countries with an IMR lower than of 7 per 1000 live births have been able to reach NBS coverage of more than 90%. Some NBS programs within the region have included expanded newborn metabolic screening on their screening panels but the number of conditions screened varies widely, including countries just beginning NBS programs and currently screening for only a single condition. This session will discuss developments in Asia for the past 2 years: (1) expanding panels and newborn screening coverage in the 24 countries; (2) ongoing pilot studies on LSDs, SMA, citrin deficiency, SCID, Fragile X syndrome, X-ALD and Wilson"s Disease; (3) obstacles in countries with low NBS coverage; and (4) network opportunities for developing programs in Asia.

I11. *The Scientific Evidence for Cchd Screening and Its Practical Application Using Pulse Oximetry*

Andy K. Ewer

Institute of Metabolism and Systems Research, Birmingham, UK

Pulse oximetry screening (POS) is a highly specific, moderately sensitive test for detecting life-threatening, critical congenital heart defects (CCHDs) that meets the criteria for universal screening.

The possibility of using pulse oximetry as a screening test was first investigated over 10 years ago and since then data from more than 370,000 screened babies have now been published. There is significant heterogeneity in published screening algorithms but almost all demonstrate that the addition of POS reduces the "diagnostic gap"—i.e., those babies with CCHD who are missed by existing screening methods and discharged from hospital before the diagnosis has been established—to less than 10%. POS also detects babies with other hypoxaemic non-cardiac conditions such as congenital pneumonia and early-onset sepsis. Although these conditions are technically false positives their early identification is a potential added benefit of screening.

Many countries have recommended routine PO screening (e.g., USA) and many more (e.g., UK) are actively considering its introduction. When considering screening at a national level the effect of the differences in screening algorithms or pathways may have a potentially significant clinical impact. This review will consider the available evidence and assesses the practical options for the introducing pulse oximetry screening including timing of screening and the parameters defining a positive test. Particular emphasis will be on the importance of a timely diagnosis for CCHD and the definition and consequences of identifying false positives.

I12. *Global Implementation Efforts in Cchd Screening Including Barriers to Implementation and Working Solutions*

Gerard Martin, Lisa Hom and Andy Ewer

Children's National Heart Institute, Washington DC and Institute of Metabolism and Systems Research, Birmingham, UK

Background: Congenital heart disease is the most common birth defect with critical forms (CCHD) affecting ~3–4 per 1000 infants. Despite routine prenatal ultrasound and clinical assessment in the immediate neonatal period 20%–50% of infants with CCHD are detected late or after discharge. Death due to late and undetected CCHD accounts for nearly 40% of infant deaths related to congenital malformations. CCHD screening using pulse oximetry in asymptomatic infants, particularly when combined with existing screening methods, can significantly narrow the diagnostic gap.

Methods: CCHD screening is conducted in asymptomatic infants before discharge from hospital of birth. Pre- and post-ductal oxygen saturations are measured by applying a pulse oximeter probe to an infant's right hand and either foot. If the infant passes the screen, normal newborn care is continued. If the infant does not pass, further evaluation including assessment and investigation including echocardiogram, if appropriate, will identify CCHD and frequently serious non-cardiac pathology.

Results: Overall sensitivity and specificity of CCHD screening based on a meta-analysis has been shown to be 76.5% and 99.9% respectively. The false positive rate is 0.05% when screening after 24 h of age, many false positives are important secondary non-cardiac conditions including sepsis and pneumonia. CCHD screening is being implemented as the standard of care in many countries.

Conclusions: CCHD screening of asymptomatic infants can improve the detection of CCHD and important secondary conditions and should be adopted globally.

I13. Developing a Point of Care Infrastructure for Cchd Screening and Nursing Considerations

Lisa Hom and Gerard Martin

Children's National Health System, Children's National Heart Institute, Washington, DC, USA

Background: In 2011, CCHD screening using pulse oximetry was added to the Recommended Uniform Screening Panel by the U.S. Secretary of Health and Human Services. The decision on whether to require the screen, and the extent to which each state department of health would be involved in surveillance and data collection, remained at the state level. Forty-eight states currently require CCHD newborn screening, with some variation in whether special populations such as home births or specialty nurseries are included. Infrastructure for point-of-care screening was less developed than bloodspot screening and is often organizationally separated. Nurses are ideally situated to contribute to development of best practices for implementation and provide education to families on CCHD screening.

Methods: Expert stakeholder work groups convened in Washington, D.C. and made recommendations on best practices for implementation, education, follow-up, and reporting. Endorsements of a common protocol by the American Academy of Pediatrics and other national organizations quickly followed. Physicians, nurses, and parent organizations engaged in advocacy and the development of toolkits, educational videos, data reporting mechanisms, and webinars to enable rapid, systematic implementation. Much of the standardization, advocacy, and development of national recommendations occurred with key input from nurses.

Results: An estimated 98% of births in the United States are now being screened for CCHD. Close collaboration between work group members and national medical associations reduced variation in screening algorithms; promoting uniform data collection, and analysis. An ongoing technical assistance infrastructure around this point-of-care screen was developed including nursing considerations such as how and when to screen, education for families and quality assurance.

Conclusions: CCHD newborn screening is now part of the recommended universal screening panel in the U.S. CCHD screening has expanded from small pilot projects in academic centers to nationwide implementation with state-focused public health oversight. Smooth implementation can be achieved by identifying champions early, obtaining input from a multi-disciplinary team including both physician and nursing leaders, and identifying ways to integrate screening into already existing workflow.

I14. Screening for Critical Congenital Heart Defects after Homebirths and Early Discharge in The Netherlands

Ilona C. Narayen [1], N.A. Blom [2], A.H.L. van Kaam [3], J.M.M. van Lith [4] and A.B. te Pas [1]

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Pulse oximetry (PO) screening for critical congenital heart defects (CCHD) has proved to be accurate and cost-effective, and is increasingly implemented worldwide. It is unknown whether PO screening is (cost)effective in settings with homebirths and very early discharge. We assess this in the Netherlands, where there is a high home birth rate and early discharge after delivery in hospital. An adapted protocol is used, fitting the working scheme of community midwives. In 2013–2014 we performed a pilot study in the Leiden region to assess feasibility in our healthcare system. Screening was performed at least 1 h after birth and at day two or three, during the midwife visit. With 3059 infants screened (99% of infants with parental consent), the false positive rate was 1.0% with significant pathology found in 62% of these cases. No CCHD was detected, nor missed. Median screening time were 1.8 h after birth and 37 h after birth. PO screening in the Dutch healthcare system was proven to be feasible. Currently, we are performing a larger implementation trial to assess the (cost)effectiveness of the study, with the aim to screen 20.000 infants. The updated results of this implementation trial will be presented at the ISNS meeting.

I15. Informing Parents and Professionals about Neonatal Screening Programs—Strategies in the Philippines

Carmencita Padilla

Newborn Screening Reference Center, National Institutes of Health, University of the Philippines Manila, Philippines

In developed countries newborn screening is a routine procedure. However in most developing countries, especially those with beginning programs or low coverage, screening uptake is affected by several factors. One of these factors is how the parents and professionals are informed about neonatal screening. The timing of provision of information on newborn screening to the mother and the rest of the family is critical—whether done prior to pregnancy, during prenatal visits or at birth of the baby. It allows for the opportune time for parents to be made aware and decide to have their child undergo screening. The strategies for informing parents and professionals in the Philippines changed with the passage of the Newborn Screening Act of 2004, the law that guides the implementation of the newborn screening program in the country. The law requires all health professionals to inform the family the benefits of newborn screening. To aid the community, the Department of Health (DOH) and the Newborn Screening Reference Center (NSRC) provide the standardized materials to all hospitals (posters); to all pregnant mothers (brochures); to the midwives, nurses and physicians (annual newborn screening conferences); and to the general public (broadsheet announcements; radio, television and cinema house commercials). In the past couple of years, the program benefited from the increased utilization of social media tools, i.e. text messaging, twitter and facebook. The DOH and NSRC continue to review materials and explore new strategies to provide better materials. The session will present examples of the various information materials of the newborn screening program.

I16. How to Inform Parents and Professionals about NBS, the Dutch Experience

Eugenie Dekkers

National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

In the Dutch newborn bloodspot screening programme the informed consent is important. This means that parents need to have enough information to make a well-considered choice about the different opportunities in the programme.

The Dutch Centre for Population Screening developed a framework for the information given in all national screening programmes which is also addressed in the newborn bloodspot screening programme.

In the Netherlands there are approximately 2.5 mln people with a low literacy. They have problems with reading and writing. For this group there is developed specific information on the newborn bloodspot screening programme. Since 2015, the Dutch newborn bloodspot screening programme is implemented in Bonaire, Saint Eustatius and Saba. For the use on the Caribbean islands there are made specific information materials. In this presentation there will also be given an overview on the materials and instruments that are developed to inform and train the professionals carrying out the NBS programme in the Netherlands.

I17. Newborn Screening in Uganda

Charles Kiyaga

Central Public Health Laboratories, Uganda Ministry of Health, Kampala, Uganda

Background: After the sickle cell survey, which depicted a high prevalence of sickle cell trait and disease in Uganda whose results have been published in The Lancet Global Health, (28 January 2016; http://dx.doi.org/10.1016/S2214-109X(15)00288-0) indicated the hot spots. We decided to start targeted newborn screening in high burden districts. 8 districts out of the 49 high burden ones were selected for the initial pilot.

Opportunities: Results of the sickle cell prevalence study, Laboratory capacity built through the study, Sample and results transport network.

Methodology: It all started with a training of health workers that was conducted at the hub. Each site that is served by that hub had to be represented by at least 2 people in the training. The training was conducted in 1 day at the hub using a comprehensive sickle cell curriculum. Trainees included physicians, nurses, lab techs, sample transport coordinators, etc. A sickle cell clinic was established at the hub.

Results: Newborn screening was initiated in 8 hubs/districts, serving over 274 health facilities. In the first 6 months over 23,531 babies had been tested, out of who 230 babies I had been identified as sicklers some of whom were put on some form of a care plan especially those identified at the hub.

Challenges and Conclusions: Personnel numbers and attitudes, Patient follow dynamics, Cultural factors, Patient follow-up challenges, Poor health manager involvement, Project vs. Program mode, Lack of an efficient patient care plan with the necessary logistics are some of the challenges. Challenges notwithstanding, Uganda being a developing country has been able to setup a newborn screening program. Sharing this experience and the lessons learned will certainly inspire similar efforts in other developing countries.

I18. Newborn Screening for Sickle Cell Disease in Africa: Public Health Meets Reality

Kwaku Ohene-Frempong

Sickle Cell Anemia Foundation, Accra, Ghana

No abstract received.

I19. 26-Hour Whole Genome Sequencing for Neonatal Genetic Diagnosis and Neonatal Precision Medicine

Stephen Kingsmore

Rady Pediatric Genomic and Systems Medicine Institute, San Diego, CA, USA

Genetic diseases and congenital anomalies are the leading cause of death in infants, neonatal intensive care units (NICU) and pediatric intensive care units in the United States. Making a timely etiologic diagnosis is critical for medical decision making, both in terms of delivery of precise medical treatment and, where the prognosis is hopeless, to help navigate decision making with regard to institution of palliative care. In 2012 we developed methods for diagnostic whole genome sequencing (WGS) in 50 h with ~96% analytic sensitivity. When used in 35 selected infants in a level IV NICU, a rate of diagnosis of 57% was achieved, compared with 9% diagnosis by conventional genetic testing. Diagnoses changed acute management in two thirds of infants, including several who received life-saving treatment or avoided major long-term morbidity. Recently we have improved these methods, achieving time to result of 26 h, ~99% analytic sensitivity, and the ability to detect structural variations. We are analyzing the results of a randomized controlled study of rapid WGS in a Level IV NICU and hope to share the results in September. We will also discuss the remaining bottlenecks to large scale implementation of rapid whole genome sequencing for infants with potential genetic diseases, and potential methods to address them.

I20. Whole Genome Sequencing in Newborn Screening? A Statement on the Continued Importance of Targeted Approaches in Newborn Screening Programmes

Heidi Carmen Howard [1], Bartha Maria Knoppers [2], Martina Cornel [3], Ellen Wright Clayton [4], Karine Sénécal [2] and Pascal Borry [5]. Endorsed by the European Society of Human Genetics; the P3G International Paediatric Platform; the Human Genome Organisation; and the PHG Foundation.

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The advent and refinement of sequencing technologies has resulted in a decrease in both the cost and time needed to generate data on the entire sequence of the human genome. This has increased the accessibility of using whole genome sequencing (WGS) and whole exome sequencing (WES) approaches for analysis in both the research and clinical contexts. The expectation is that more services based on these and other high-throughput technologies will become available to patients and the wider population. Some authors predict that sequencing will be performed once in a lifetime, namely shortly after birth. The Public and Professional Policy Committee of the European Society of Human Genetics (ESHG), the Human Genome Organisation Committee on Ethics, Law and Society, the PHG Foundation, and the P3G International Paediatric Platform address herein the important issues and challenges surrounding the potential use of sequencing technologies in publicly funded newborn screening (NBS) programmes. This Statement presents the relevant issues, including the potential pros and cons of using a high-throughput approach in NBS, and culminates in a set of recommendations to help inform and guide scientists and clinicians, as well as policy makers regarding the necessary considerations for the use of genome sequencing technologies and approaches in newborn screening programmes. The main point all readers should retain is that the primary objective of NBS should be the targeted analysis and identification of gene variants conferring a high risk of preventable or treatable conditions, for which treatment has to start in the newborn period or in early childhood.

I21. Problems Associated with Dried Bloodspot Analysis

Christophe Stove

Laboratory of Toxicology, University of Ghent, Ghent, Belgium

Analysis of dried blood samples brings along some challenges when it comes down to reporting quantitative results. Ideally, there should be no difference when blood is analyzed in either a liquid or dried blood format. This presentation will focus on the issues and challenges associated with the collection and analysis of dried blood samples. These include volume and hematocrit effects, spot inhomogeneity, issues related to correct sampling, contamination and stability, as well as how to interpret a blood concentration in the context of plasma reference values.

I22. Approaches and Technologies Offering Potential Solutions to the Problems Associated with Dried Bloodspot Analyses

Neil Spooner

Spooner Bioanalytical Solutions Ltd., Hertford, UK

This presentation follows on directly from that of Christophe Stove. It will outline a number of currently available and emerging technologies to help overcome the potential errors identified in dried blood spot analysis. Further, the practicalities of using these technologies and factors to consider when deploying them will be discussed, with the endpoint being the collection, shipment, storage and analysis of samples to give data quality suitable for decision making.

I23. The Automated Screening Laboratory of the Future, What do We Want

Kate Hall

West Midlands, UK

Automation in the neonatal screening laboratory has transformed many previously laborious and error prone repetitive tasks. Covering steps from recording babies' demographics, automated punching and extraction, to fully automated analysis, as well as automated result generation and reporting, this presentation will consider opportunities to improve robustness in the screening process and to reduce hands on time.

I24. Monitoring and Evaluation of Newborn Screening Programmes

Paul H. Verkerk

TNO, Department of Child Health, Leiden, The Netherlands

Newborn screening programmes are extremely valuable. In general they deliver far more good than harm at reasonable cost. However, this will only be the case for those programmes that achieve a good quality. If quality would drop below a certain level it is possible that the amount of harm will be greater than the amount of good. In the Netherlands a monitoring program is in place to ensure that quality is maintained or even improved. Newborn screening has three objectives: program sensitivity should be high, patients should be detected early and the amount of harm should be as low as possible. In the Netherlands laboratory quality is monitored by the RIVM, other aspects are monitored by TNO. Due to our monitoring program several quality indicators have gradually improved over the years. For instance, the average age at which patients with a severe form of CH were treated in the early 1980s was 20 days, whereas in more recent years these patients were treated at the average age of 7 days.

Conclusion: a monitoring program should be in place for every neonatal screening program to guarantee that screening will do more good than harm.

I25. Quality Indicators for Continuous Improvement in a Newborn Screening Program

Scott Shone

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Newborn screening (NBS) is a public health program that entails many components including testing, diagnosis, follow-up, treatment, education and evaluation, including continuous quality improvement (CQI). Routine monitoring of quality indicators (QIs) that encompass the entire NBS System from pre-analytic through post-analytic activities are an integral part of the CQI process. In the United States, the Newborn Screening Technical assistance and Evaluation Program (NewSTEPs) adopted a set of QIs, which were developed and refined by stakeholders from the newborn screening community. Use of these standardized QIs permits longitudinal evaluation of NBS Programs as well as comparisons between Programs.

I26. The Telephone Rings for a Positive Nbs Result for One out of >20 Metabolic Diseases: Diagnostic and Logistic Challenges in The Netherlands

Francjan J. van Spronsen on behalf of the Advisory Board for Neonatal Screening for Inherited Metabolic Diseases, University Medical Centre Groningen, Groningen, The Netherlands

In the Netherlands NBS for inherited metabolic diseases (IMD) was only on PKU till 2007, while the benefits of NBS for MCAD was investigated as a pilot. In 2005 a report of the Dutch Health Council was published distinguishing three categories for NBS: disorders for which considerable irreparable damage can be prevented (category 1), disorders for which this applies to a lesser degree or for which the evidence is inconclusive (category 2), and disorders for which NBS does not prevent damage to health (category 3). Only diseases of category 1 were included: Biotinidase deficiency (BD), Galactosemia (GAL), Glutaric aciduria type I (GA I), Holocarboxylase or multiple carboxylase deficiency (HCL), Homocystinuria (HCY), 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG), Isovaleric acidemia (IVA), Long-Chan-Acyl Co A Dehydrogenase deficiency (LCHAD), Maple syrup urine disease (MSUD), Medium-chain acyl-CoA dehydrogenase (MCAD); 3-methylcrotonylcarboxylase deficiency (3-MCC), PKU, Tyrosinemia type I (TYR I), Very Long Chain Acyl CoA Dehydrogenase deficiency (VLCAD).

Within 3 months after the introduction of the extended NBS, the program had to be changed: NBS for TYR I (based on tyrosine levels) was put on hold, the levels to refer a child for a positive NBS result for Galactosemia had to be adapted due to the very high number of false positives. Some months later discussion started on NBS for HCY, which was later put on hold and now has been stopped. All children with a positive NBS for IMD were referred to one of the 8 (later 7) university medical centers for diagnostics and care within some hours and were generally seen within 24 h (with exception for BD in case of "high" levels). Two years ago, the cut off for referral of BD (30%) was lowered to 20%, and probably will be lowered again shortly. The cut off for phenylalanine for PKU was decreased from 240 to 200 μM, but this was due to conversion rates of the NBS measures; 200 μM in NBS being 240 μM in the metabolic laboratories. In the NBS 14 IMD are included, but still children appear to have also Multiple acyl-CoA dehydrogenase deficiency; trifunctional protein deficiency, pterin defects, OCTN2, and IMD in the mothers such as 3-MCC, GA-I, MCAD, PKU. Discussion is ongoing on some important questions that will be addressed during the presentation. 1. Should moment of blood sampling not be antedated from 72 (96)–168 h to 48–120 h? This is hampered by the time you may indicate birth of a child at the civil registry and the fact that the heelprick is combined with the screening for hearing defects (usually >96 h); 2. Where does the process of screening end? After the referral call of the medical advisor of the screening organization, at the door of the hospital or after the diagnosis of true or false positive is made? This is a political decision where curative care and public health; 3. How to guarantee good studies in follow-up to know whether NBS is effective? Addressing this question is at least hampered by the fact that the diversity of IMD is large and the incidence is low. Questions only partly can be addressed in the same way for all IMD; 4. Do we find patients or also children with biochemical findings that do not need very careful follow-up let alone treatment? This is largely hampered by at least the same issues as in Q3.

I27. Diagnosis and Treatment of Congenital Hypothyroidism

Annette Grüters

Charité University Hospital, Berlin, Germany

More than forty years ago screening for congenital hypothyroidism (CH) has been started. Although unexpected in the 1970s, early thyroid hormone replacement can rescue even children without a thyroid gland from mental retardation and it should be the goal of any screening program to establish thyroid hormone replacement as early as possible in children affected by hypothyroidism with low peripheral thyroid hormone levels. With the aim to detect also mild forms of CH has resulted in a continuous lowering of the TSH cut-off level from >50 mIU/L in the 1970s and 1980s to in some countries as low levels as >5–8 mIU/L in 2015. This has led to an increase in the detection of patients with hyperthyrotropinemia, normally developed glands and mostly normal or slightly decreased T4 levels, while the frequency of patients with thyroid dysgenesis remained constant. So far there is no evidence, that children with TSH elevations will benefit from the detection by newborn screening. These changes of screening strategies are not in accordance with the Wilson & Jungner criteria, because the natural course of the newly detected conditions is unknown. In contrast only few countries include the measurement of T4 in screening and cases of central hypothyroidism are being missed, although there are cases of central hypothyroidism, e.g., specific ß -TSH mutations or LHX3 mutations, which lead to severe hypothyroidism and mental retardation. Therefore, instead of lowering the cut-off levels of TSH, it is time to consider strategies for newborn screening to detect also patients with central hypothyroidism, which will suffer from mental retardation, if therapy is delayed.

I28. Diagnostic Challenges Associated with Screening Newborns for CF

Kevin W. Southern

University of Liverpool, Liverpool, UK

There are some considerable challenges that face the laboratory and the clinic after a positive newborn bloodspot screening (NBS) result. These challenges relate to (1) the spectrum of clinical phenotype associated with mutation of the CFTR gene; (2) the sensitivity of the IRT assay for identifying CFTR dysfunction; (3) the health service structure for processing results (4) the issue of carrier recognition and (5) the practical of issue of sustaining a sweat test service for a challenging population (small babies) and when paediatricians are referring less infants for sweat testing. This lecture will consider these underlying themes and the factors that impact on these. These confounding factors include laboratory and clinical approaches, as well as infrastructure. Finally the situation of an unclear diagnosis will be considered, both the new designation for these infants, CF Screen Positive, Inconclusive Diagnosis (CFSPID) and an approach to diagnosis and early management.

I29. Screening from a Family Viewpoint—Expectations, Aspirations and Unforseen Consequences

Alastair Kent

Genetic Alliance UK, London, UK

In this talk I will explore the issues generated for families by the availability of screening. I will discuss the issues for parents with no obvious family history of risk, and from the perspective of those who know they might be carriers of a mutation creating a risk of having a child with a life limiting genetic disease. A central issue is what couples know, and what they think that the results of a screening programme might be able to tell them. Attitudes to new knowledge and the way a diagnosis resulting from a screening programme changes lives for ever, in ways that can be empowering or devastating, depending on the couple, their residence and the support they receive in getting a diagnosis. I will also look at the potential impact of new technologies such as NGS and NIPD for women and couples.

I30. Newborn Screening in Latin American Countries: Lessons Learned and Challenges

Pablo Duran

Latin American Center of Perinatology, Montevideo, Uruguay

No abstract received.

3. Oral Presentations

O01. Dried Blood Spot Contamination—An Underestimate Risk in Newborn Screening

Theresa Winter, Anja Lange, Matthias Nauck and Cornelia Müller

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Objectives: Filter paper with dried blood is the standard specimen used for newborn screening all around the world. The convenient transportation by regular mail and the reasonably stability of metabolites of interest support its use. The heel pick procedure as the method of sample acquisition is well standardized. The use of native blood without anticoagulants (e.g., EDTA) is required since they are known to interfere with screening laboratory methods.

However, other invisible contaminations of the filter paper prior, during or after the sample collection are often not visually detectable and do have, nevertheless a significant influence on the screening result. In order to emphasize the correct pre-analytical phase within the Newborn screening, eight different contamination sources, which are present in our neonatal ward, were evaluated in terms of their impact on the screening result.

Methods: Capillary blood was obtained from 10 volunteers and applied on screening filter papers. Afterwards, separate spots were intentionally contaminated with either (1) disinfectant; (2) feces; (3) urine; (4) baby cream; (5) baby food; (6) ultrasonic gel; (7) breast milk and (8) baby wipes. One spot was left out of any contamination for control purposes.

TSH, 17-OHP, GALT, Biotinidase, IRT, amino acids and acylcarnitines were analyzed.

Results: All contaminants affected in some way the analysis. Most pronounced were the false positive effects of feces on IRT, of urine on the Glutaric acidemia type I analyte C5DC and baby cream on the GALT activity.

Conclusion: Mishandling of filter papers in the pre-analytical phase is a source of false positive and false negative screening results and underlines the importance of regular training of the medical staff involved in the screening process.

O02. A Novel Non-Contact Method to Determine the Hematocrit of Dried Blood Spots

Sara Capiau, Leah Wilk, Maurice Aalders and Christophe Stove

Ghent University, Laboratory of Toxicology—Faculty of Pharmaceutical Sciences, Ghent, Belgium

The hematocrit (Hct) effect is considered to be one of the most crucial issues in dried blood spot (DBS) analysis. Since the Hct of a blood sample affects blood viscosity and hence, the volume of blood contained in a fixed-size DBS punch, deviating Hct values can significantly impact DBS-based quantitation. To evaluate the extent of the Hct effect for a given DBS we previously developed a method that allows to estimate the Hct of a DBS based on its potassium (K+) content [1]. Additionally, using caffeine and paraxanthine as model compounds, it was shown that the K+ content could also be employed to introduce a Hct specific correction factor (utilizing a K+-based correction algorithm) which alleviates the Hct effect [2].

Although this K+-based method yielded good results when applied to patient samples, it also suffered from some practical drawbacks, as it consumed part of the DBS and required additional sample preparation. Therefore, we now developed a non-destructive method which allows to predict a DBS' Hct using non-contact diffuse reflectance spectroscopy. This way, mere scanning of a DBS suffices to derive its Hct. The non-contact method was successfully validated and applied to 233 patient DBS

with varying Hct values (Hct = 0.20–0.50). The Hct of these patient DBS was estimated using the non-contact method, whilst the true Hct of the patient samples was determined on the corresponding venous whole blood samples using a Sysmex XE-5000 hematology analyzer. The non-contact method was found fit for purpose, since a good correlation was observed between the estimated and true Hct (r = 0.95) and since only 4.72% of the estimated Hct values deviated more than 15% from the corresponding true Hct values.

Reference in O02.

1.　　Capiau; et al. *Anal. Chem.* **2013**.
2.　　De Kesel; et al. *Anal. Bioanal. Chem.* **2014**.

O03. Improved Procedure for Obtaining Lyophilized Phenylalanine Free Serum Using Phenylalanine Ammonia Lyase

Gustavo Borrajo, María Teresita Castaneda and Roque Hours

Fundacion Bioquimica Argentina, Deteccion de Errores Congenitos, La Plata, Argentina

Background: Considering the unavailability of phenylalanine (Phe) free human blood, we have recently described a procedure for obtaining low Phe serum to be used in the preparation of reference materials and calibrators for newborn screening (NBS), using L-Phenylalanine Ammonia Lyase, (PAL, EC 4.3.1.25). (JIEMS. doi:10.1177/2326409815613261. 2015).

Objective: To present the recent modifications introduced to the original protocol for obtaining low Phe serum and their corresponding results.

Methods: PAL from Rhodosporidium toruloides (NBRC 0559) catalyzes the non-oxidative deamination of L-Phe producing trans-cinnamic acid (t-CA). The original protocol consists in treating a normal serum pool with PAL immobilized on calcium alginate beads (483 mU/mL of reaction mixture) at 37 °C for 5 h, and the following recovery of the serum without addition of exogenous substances. In the modified procedure, PAL activity was increased twice (966 mU/mL), the incubation time was reduced at 90 min, and most of the t-CA produced was eliminated by centrifugation after keeping the serum overnight at 2–8 °C. Phe was measured by Tandem Mass Spectrometry. The resulting serum was freeze dried and kept at room temperature.

Results: The modified procedure allowed obtaining an almost free-Phe serum, with a negligible final concentration lower than 0.4 μmol/L. The serum lyophilization allowed to have a time-stable material easy to be transported and suitable to be used in the preparation of Phe reference materials and calibrators for NBS.

Conclusions: The obtained results indicate that the freeze dried Phe-free serum is an appropriate material for the intended purpose. The modified procedure demonstrates to be reproducible, inexpensive and easy to perform, offering a new alternative to improve the assignment of Phe values to reference materials and calibrators provided for commercial reagent kits.

O04. Evaluation of a Digital Microfluidic Platform for Newborn Screening of Four Lysosomal Storage Diseases: Preliminary Results of a Pilot Project

Eurico Camargo Neto, Jaqueline Schulte, Jamile Pastorello, Claudio Sampaio Filho and Roberto Giugliani

Centro de Triagem Neonatal, Laboratorio Nobel Rie, Porto Alegre, Brazil

Newborn screening for lysosomal storage diseases (LSDs) has been gaining considerable interest as a result of the new brazilian policy to promote early diagnosis of rare diseases, availability of new screening methods and enzyme replacement and other specific therapies. We present the preliminary results obtained with a fluorometric digital microfluidic platform (Baebies, Inc.) to perform multiplexed enzymatic analysis of α-L-iduronidase (IDUA) to screen for MPS I; acid α-glucosidase (GAA), for Pompe; acid β-glucosidase (GBA), for Gaucher and acid α-galactosidase (GLA) for Fabry diseases in 8.550 dried blood spot (DBS) samples randomly selected for testing among the cards routinely received by the Neonatal Screening Center, based in Porto Alegre, Brazil. The method

correctly discriminated 9 samples of affected patients (3 MPS I, 1 Pompe, 2 Gaucher and 2 Fabry) from cases previously diagnosed and one sample detected in the routine with very low activity IDUA (0.8 μmol/L/h; cutoff = 5.0) later confirmed as a pseudo MPS I. The protocol of the assay to determine the enzyme activity in a multiplex format was less than 4 h. Overall coefficient of variation (CV) values between cartridges, days, instruments, and operators ranged from 4% to 20%; linearity correlation coefficients were ⩾0.98 for all assays. Digital microfluidic technology shows potential for rapid, high-throughput screening for 4 LSDs in a standard newborn screening laboratory.

O05. *Harmonisation of MSMS Results Used in Newborn Screening: A Practical Solution?*

Rachel Carling, Finlay Mackenzie, Kate John and Jim Bonham

Viapath, South East Thames regioanl Newborn Screening Laboratory, London, UK

The NHS newborn bloodspot screening program uses nationally agreed screening protocols with specified cut-off values (COVs) so harmonisation of results across the 14 screening labs is important. Most labs use in-house MSMS methods with quantitation based on isotope dilution alone. The measurement uncertainty associated with these results is ±20% to ±36% at concentrations close to the analytical COVs and the Horwitz ratio indicates analytical performance of some analytes is suboptimal. While false negative results are unlikely, there is potential for false positives causing unnecessary stress to families.

The aim of this study was to determine if harmonisation could be improved by use of a common set of calibrators or a common internal standard (IS) mix. 16 labs performed analysis of key analytes on bloodspot samples ($n > 500$) using established in-house methods. Calibrators ($n = 6$) prepared by UKNEQAS were also analysed. The exercise was repeated using a centrally prepared IS mix. Population centiles were determined for the 3 sets of results from each lab, for 8 analytes.

Surprisingly, a common set of calibrators did not improve the harmonisation of results. However, the interlab variation was significantly reduced ($p < 0.05$) when the centrally prepared IS mixture was used. The range of 90th centiles reported for leucine (μmol/L) varied from 131–262 for the calibrated method, 186–278 for the in-house method and 157–228 for the common IS mix.

Achieving harmonisation between laboratories is challenging due to differences in instrument set up, lack of certified reference materials and calibrators and the financial implications associated with commercial kits. The central distribution of a common IS mix may be a cost effective solution to support the use of a common national COV.

O06. *International Differences in the Evaluation of Conditions for Newborn Bloodspot Screening: A Review of Scientific Literature and Policy Documents*

Marleen Jansen, Selina Metternick-Jones and Karla Lister

School for Oncology and Developmental Biology (GROW), Faculty of Health, Medicine, and Life Sciences, Maastricht University, Institute for Public Health Genomics, Maastricht, The Netherlands

Despite international adoption of newborn bloodspot screening (NBS), no two countries' screening programs are the same. This review aims to understand what factors influence NBS criteria and how condition are assessed against them, and offers unique insights into the international landscape of NBS.

A systematic review on NBS criteria in scientific literature was undertaken first. Through this, five topics were identified for consideration when analysing NBS decision making. Using these five topics as a template, a structured analysis was conducted on NBS in policy documents of eight countries.

Programs are using different approaches to explore the same policy issues, including: the beneficiary of NBS, definition of criteria, the way conditions are assessed, level of evidence required, and recommendations after assessment. These differences have the potential to result in increased disparity across NBS internationally. Ultimately, governments need to decide on their role and an approach to NBS decision making in line with this role.

The analysis presented in this article highlights that despite programs" commonalities, no one "NBS decision making solution" exists. Understanding the different approaches to decision making within the literature and policy settings, provides an objective lens to inform decision making approaches and the future direction for NBS programs.

O07. Health System Impact of Positive Newborn Screening Results

Christine Davies, Sara D. Khangura, Maria D. Karaceper, Pranesh Chakraborty, Doug Coyle, Kumanan Wilson, Robin Ducharme, Steven Hawken and Beth K. Potter

Children's Hospital of Eastern Ontario, Newborn Screening Ontario, Ottawa, ON, Canada

The health system impact of newborn screening (NBS) is poorly understood. We used NBS and health care data in Ontario, Canada to examine health services use among infants with true positive NBS results (study 1) and those with false positive results (study 2). We focused on: medium-chain acyl-CoA dehydrogenase deficiency (MCADD), phenylketonuria (PKU), and sickle cell disease (SCD, study 1 only).

Eligible infants had NBS in Ontario from 2006–2010 (MCADD & SCD) or 2006–2012 (PKU). We compared incidence rates of emergency department (ED) visits, hospitalizations and physician contacts from birth to 2012/2013 (MCADD & SCD/PKU) between screen positive groups and screen negative controls. Rate ratios (RRs) were adjusted for sex, gestational age, birthweight, socioeconomic status, and urban/rural residence.

In study 1, all three groups had higher rates of hospitalizations (RRs: MCADD, 8.39 [95%CI 5.18–14.22]; PKU, 6.15 [3.88–9.90]; SCD, 17.07 [14.93–19.56]) and physician contacts (RRs: MCADD, 1.42 [1.20–1.71]; PKU, 1.49 [1.29–1.74]; SCD, 1.95 [1.79–2.12]) relative to controls. Infants with MCADD (RR = 2.02 [1.47–2.87]) and SCD (RR = 3.91 [3.33–4.63]) had elevated ED visit rates relative to controls. In study 2, infants with false positive NBS results for MCADD or PKU had higher rates of hospitalizations (RRs: MCADD, 1.57 [0.82–3.01]; PKU, 5.09 [3.80–6.86]) and physician contacts (RRs: MCADD, 1.22 [1.03–1.46]; PKU, 2.97 [2.71–3.26]) relative to controls.

Children with MCADD, PKU, or SCD had higher rates of health services use relative to controls. Children with false positive NBS results for MCADD or PKU had higher rates of some health services relative to controls, which may be explained by underlying health differences or health care use in response to the screening result itself.

O08. How Small Is Small? Rethinking Risk for Newborn Screening Refusals

Jeremy Penn

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Participation in newborn screening exists on a broad continuum from countries where newborn screening is not widely available to countries or states where screening is compulsory with no provision for parental refusal. Policy decisions regarding the compulsory nature of newborn screening are complex and full of ethical issues. Justification for keeping newborn screening non-compulsory typically emphasizes the low level of risk of developmental delay, morbidity, or death for an individual child who is not screened. However, as more conditions have been added to newborn screening panels the perceptions of risk have not changed accordingly. The purpose of this session is to describe data from the United States on the risks for refusal to determine whether or not this increased level of risk warrants intervention.

To begin I will use the Wilson and Jungner (1968) principles to examine the context for refusal of newborn screening in the United States and the arguments both for and against compulsory newborn screening.

Next, I will use data from the United States to show how expanded newborn screening to more than 30 conditions has dramatically changed the level of risk for refusals. Using a population

perspective, the presenter will show how even a small number of refusals (1%–2%) represents a significant and important health risk to infants.

In closing, I will examine the ethical issues that arise when considering strategies for addressing the problem of refusal of newborn screening. States and countries have a range of policy options and a range of strategies to consider when making decisions about refusal policy. The presenter will provide a framework for thinking about these issues and identify implications for policy, providers, health departments, and parents, families, and infants.

This presentation is critical for both the ISNS, as the ISNS Guidelines (Section 4.3) recommend a provision for parents to refuse newborn screening, and for newborn screening programs around the world as we seek to prevent unnecessary morbidity and mortality through newborn screening.

O09. National Program of Newborn Screening for Congenital Hypothyroidism in Morocco, Situation and Prospects

Laila Acharai

Ministry of Health, Protection of Mother and Neonatal Health, Rabat, Morocco

Context: Congenital hypothyroidism can cause mental impairment and growth retardation in newborns. Early diagnosis through newborn screening and treatment of hypothyroidism within the first few weeks of life can prevent the complications of this disorder. In Morocco, since 2012, a National Newborn Screening Program (NNBS) for congenital hypothyroidism has been established by the ministry of public health. To implement the NNBS for congenital hypothyroidism, three main phases were defined: 1- pilot phase, 2-expansion phase, 3-nationwide.

Results: The pilot phase was launched from 5 March 2012, in seven public health care structures all of which represented 80% of expected births in the pilot site. Health professionals and laboratory technicians were trained in sampling techniques, routine analysis and diagnosis confirmation. Equipment and reagents have been acquired, choice the method DELFIA for analysis, a manual guide in support of newborn screening activities directed at health care professionals has been developed. Educational materials have been developed. A monitoring system has been set up with monthly records. Data analysis of the pilot phase established a prevalence of 1 births with congenital hypothyroidismover1,613 live births. The implication of greatly motivated multidisciplinary team resulted in a clear improvement in the quality of the NNBS program with progressively reduced time transfer of samples between the health delivery unit and the analytical laboratory. Involvement of careers was increasing progressively and effectively. Diagnosed cases had subsequently a hormone replacement therapy, medical monitoring. Parental awareness about neonatal hypothyroidism increased as well.

Conclusion: Actually the program has been expanded to three new regions and an action plan has been established to implement the program nationwide and equip all regional laboratories to cover 50,000 live births per laboratory per year.

Keywords: neonatal new born screening, handicap, prevention, delfia analysis, equipment, laboratory multidisciplinary team.

O10. Newborn Screening for Primary Immunodeficiency Using Trec And Krec—A Two Year Follow-Up

Lennart Hammarström, Ulrika von Döbeln, Stephan Borte, Michela Barbaro, Annika Ohlsson, Rolf Zetterström and Jacek Winiarski

Karolinska Institutet, Clinical Immunology, Stockholm, Sweden

Newborn screening (NBS) for primary immunodeficiency was carried out in a pilot program in the Stockholm county for two years, encompassing close to 60,000 children. T cell receptor excision circles (TREC) and kappa receptor excision circles (KREC) were measured simultaneously, using a PCR

based method on DNA extracted from routinely, consecutively collected Guthrie cards, using beta-actin as a positive control for the amount of DNA.

Altogether 64 children were recalled for follow up due to low TREC and/or KREC levels and three patients with immunodeficiency were identified. The first child (Artemis SCID) has already been successfully transplanted. The additional positive samples were mainly due to prematurity and genetic aberrations. Seven children, born to mothers treated with Azathioprine during pregnancy, showed low KREC levels that however normalized spontaneously after 2–6 weeks of follow-up. Only three children were referred to FACS analysis.

During the screening period, 16 triplets and 896 twins were born and analysis of the concordance of TREC/KREC is ongoing as is the evaluation of the results from the 3,469 prematurely born children (born before gestational week 37).

This is the first comprehensive study with a simultaneous detection of both TREC and KREC, allowing newborns with both T and B cell defects and may thus serve as a basis for a subsequent national implementation of this test in Sweden.

O11. Integration of SCID Screening into the Dutch Newborn Screening Program: Benefits and Shortcomings of the Available Screening Assays

Blom M., Ingrid Pico-Knijnenburg, Marja Sijne-van Veen, Anita Boelen, Robbert Bredius, Mirjam van der Burg and Peter Schielen

National Institute for Public Health and the Environment, IDS-Reference laboratory Neonatal Screening, Bilthoven, The Netherlands

Introduction: Severe combined immunodeficiency (SCID) comprises a group of genetic disorders resulting in a severe cell-mediated and humoral immunodeficiency. Infants with SCID present with life-threatening infections during the first months of life and face a fatal outcome unless their immune system is replaced by hematopoietic stem cells transplantation (HSCT). Newborn SCID screening is based on the detection of T-cell receptor excision circles (TREC) in heel prick blood. B-lymphocyte deficiency-related SCID can be detected by the quantification of ƙ-deleting recombination excision circles (KRECs). In this study, we provide first experiences with two available detection methods.

Material and Methods: With the EnLite Neonatal TREC assay (PerkinElmer) and end-point PCR, 1295 fresh heel prick cards of the Dutch newborn screening program and heel prick cards stored for two weeks ($n = 61$), one month ($n = 63$), three months ($n = 33$) and one year ($n = 33$) were analyzed. Moreover, 155 heel prick cards of preterm infants, nine TREC reference dried blood spots from the CDC and peripheral blood spots of 19 confirmed and 30 potential SCID patients were included. With the SCREEN-ID assay (TRM Leipzig) and real time-qPCR, five fresh heel prick cards, seven cards of confirmed SCID patients and 18 cards of potential SCID patients were analyzed.

Results: The cut-off level, based on the distribution of TREC-levels in the 1295 fresh heel prick cards, was 39 copies/µL. A significant reduction in TREC levels was observed in heel prick cards stored for three months and one year. Preterm infants showed significant lower TREC levels and a high retest-rate than full-term infants. Finally, all 19 confirmed SCID-patients showed undetectable or low TREC levels.

Discussion: This study showed that the EnLite Neonatal TREC assay is a suitable method for SCID-screening in the Netherlands. Consequently, these findings will provide guidance in the decisions that have to be made during the incorporation process of SCID in the Dutch screening program.

O12. The Impact of a 2nd Tier Blood-Spot Methylmalonic Acid (MMA) Using Tandem Mass Spectrometry (MS/MS) on Routine Newborn Screening for Inborn Errors of Metabolism (IEM)

Enzo Ranieri, Rosemarie Gerace, Bronwen Bartlett and Janice Fletcher

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A 2nd tier (reflex-test) blood-spot test for MMA was added to the South Australian newborn screening programme in 2007 as a specific marker for a group of IEM collectively known as methylmalonic acidemias. The MMA reflex test was introduced due to the low predictive value of C3-carnitine for identifying disorders of cobalamin metabolism and to the false positives due to prematurity and hyperbilirubinaemia in neonates.

A simple non-derivatised blood-spot LC-MS/MS MMA method using a 5×100 mm Phenomenex C6-phenyl column with acetonitrile:water:formic acid at a flow rate of 150 µL/min directly into an API4000 MS/MS (SCIEX) operated in negative ion mode. MMA was eluded from a 3 mm blood-spot and determined against (d3)-MMA using MRM pairs of 117.1/73.1 & 120.1/76.1 in a 5 min isocratic LC run. A MMA level of 3.3 µmol/L whole blood equivalent to the 99th centile is used as an action limit.

Since 2007 over 1135 dried blood-spot MMA determinations have been performed as a result of a primary elevation in C3-carnitine, in addition to the related ratios C3/C2, C3/C16 & C3/methionine. From the screened population this represented <0.01%. To date, of the 1135 MMA determinations 104 babies required further follow-up either for a repeat blood-spot collection or recalled for plasma & urine MMA and B12 determination at an average age of 21 days. We have identified 26 neonates with significant B12 deficiencies and who have been treated representing ~25% of the recalled neonates. A case of cobalamin A deficiency (cblA; p.R145X/p.D292V)) was identified with a presenting MMA level of 18.3 µmol/L whole blood. In addition we have identified maternal B12 deficiency, as a result of an elevated blood-spot MMA in her baby.

Inclusion of a 2nd tier MMA identifies B12 deficiencies as well as disorders of cobalamin metabolism while significantly reducing the false positive rate associated with the measurement of C3-carnitine.

O13. A Novel Method for Inclusion of Urea Cycle Disorders into Newborn Screening

Ralph Fingerhut, Susanna Sluka, Johannes Häberle, Mia Halme and Géraldine Carrard

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The inclusion of urea cycle disorders (UCD) detection into newborn screening (NBS) is highly desirable; however it is hampered by the lack of a specific marker for most of these disorders. So far, the common feature of UCDs, hyperammonemia, is not directly detectable in dried blood spots (DBS). The detection of secondary elevations of glutamine seemed so far not feasible based on the assumption of the instability of glutamine in DBS. We describe here a reliable method for the simultaneous detection of lysine and glutamine from DBS in multiple reaction monitoring (MRM) with a second-tier ultra-high performance liquid chromatography-(UPLC)-method for the separation and specific quantitation of glutamine. We combined this newly developed method with the measurement of all specific amino acids (arginine, arginino succinic acid, citrulline, ornithine, and proline), N-acetyl-glutamate, and orotic acid. This combination proofed to be a reliable and sensitive method for the detection of all UCDs by tandem-mass spectrometry NBS. The next step will be a prospective study with dried blood samples from patients with hyperammonemia, for further testing of the method.

O14. Long-Term Outcome of Newborn G6pd Screening Program in Taiwan

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Severe neonatal jaundice (NJ) triggered by environmental factors and/or medications is the major health impact of Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency in newborns. If not prevented or treated properly, it may lead to kernicterus and cause death or permanent neurological damages. The incidence of G6PD deficiency in Taiwan is about 2%. It has been found that 30% of NJ admitted to hospital was G6PD deficient with 16% mortality and 32% developed kernicterus in 1970s.

The nationwide newborn G6PD screening program in Taiwan was started in 1987 and the coverage rate has reached >99% since 1996. The effectiveness of this screening program to prevent mortality and sequela of NJ is studied.

The patient data of hospital admission with NJ after discharged from the birthing facility between 2000 and 2010 were retrieved from the National Health Insurance Research Database, which covered >98% population of Taiwan. There were 8,635 NJ (0.38%) admissions from 2,297,867 live births and 14 of them treated with exchange transfusion. Only 2 of the NJ cases dead within 1 month of age and 8 of them developed kernicterus not due to isoimmunization. The average immediately severe morbidity and mortality were about 1 (0–2) case per year nationwide. Long-term follow up those NJ cases born between 2000 and 2004 up to 6 years old have found a higher risk of developmental delay, hearing loss, speech disorders, attention deficit hyperactivity disorder (ADHD), and mental retardation comparing to the control cohort.

The results indicated that the newborn G6PD screening program in Taiwan almost eliminated severe morbidity and mortality caused by NJ with G6PD deficiency after discharge from birth facilities. However, close follow-up of those cases with NJ admission are still needed for early intervention of developmental delay, mental disorders, hearing loss, and speech problems.

O15. Pseudo-Deficiency of Alpha-L-Iduronidase: A Challenge Faced in the Newborn Screening for Mucopolyssacharidosis I

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Mucopolysaccharidosis I is one of the new targets for NBS. Babies identified with the severe form of MPS I should be considered candidates for HSCT, and the ones with the attenuated form are considered for ERT, with firm evidences on benefits of early treatment. There are several platforms available for MPS I screening, and we ran a pilot project using the digital microfluidics technology (Baebies, Inc.). The α-L-iduronidase (IDUA) activity was determined in 8550 babies, randomly selected among the ones referred to CTN for routine NBS. One sample showed low IDUA activity (0.8 μmol/L/h, with cut off = 5.0). The IDUA activity in this sample was tested also by manual fluorimetry (undetectable) and by TMS (undetectable). The baby was retrieved and urine and blood samples were collected. Urinary GAGs were normal by standard DMB method (197 μg/mg creat, normal range = 133 to 460 μg/mg creat) and GAG electrophoresis showed a normal pattern. IDUA activity was normal in plasma (11 nmol/h/mL, normal range = 6.6 to 34 nmol/h/mL) but slightly reduced in leucocytes (11 nmol/h/mg prot, normal range = 27–171). Molecular analysis of the IDUA gene allowed the identification of the mutation {c.251G>C [p.(G84A)} in one allele, predicted as possibly pathogenic by Poly-Phen 2, SIFT and Provean. In the same codon where already described two pathogenic mutations (p.G84E and p.G84S). A genetic variation previously associated with pseudo-deficiency was found in the other allele {c.246C>G [p.(H82Q)]}.This comprehensive evaluation allowed us to predict a clinically normal child, as the residual activity provided by the p.(H82Q) allele allows a normal degradation of GAGs. This case illustrates the challenges faced by NBS of LSDs, with the need of an comprehensive algorithm to properly manage the cases when a screening test results positive.

O16. Full Population Screening for Pompe, Fabry, Gaucher, Mucopolysaccharidosis Type I and Krabbe Disorders in the State of Missouri

Patrick Hopkins

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Since 2013, the Missouri State Public Health Laboratory (MSPHL) has been using Baebies' digital microfluidic multiplex assay to screen for 4 lysosomal storage disorders (LSDs): Pompe, Fabry, Gaucher and Mucopolysaccharidosis Type I (MPS I). As part of the full population screening, all newborn screening samples received by MSPHL during this time were tested for LSDs. In 3 years of screening, the state has screened over 237,000 births and determined the following incidence rates: 1:10,300 for Pompe; 1:3390 for Fabry; 1:59,250 for Gaucher and 1:118,500 for MPS I. These incidences do not include several confirmed genotypes of unknown significance which must also be continually followed to watch for possible late onset forms of the disease that have not previously been described. Missouri's incidence rates are generally in agreement with recent published incidence rates from global population wide studies. All of the screen positive samples were confirmed through confirmatory testing and the patients were referred to one of 4 state contracted referral centers for follow-up and treatment. In late 2015, MSPHL added independent testing for a 5th LSD, Infantile Krabbe disease, through a fluorimetric assay. In 7 months of testing, Missouri has screened over 45,500 births for Krabbe disease and has found 7 newborns positively identified to be heterozygous carriers of the 30 Kb deletion of the Krabbe gene, but none to date that are affected with Infantile Krabbe disease. The newborn screening population study in Missouri is the first full population newborn screening study in the United States for four of the five LSDs. With the recent additions of Pompe and MPS I to the Recommended Uniform Screening Panel, individual states can look to Missouri as a model for population wide screening of these LSDs.

O17. *Implementation of Newborn Screening for Duchenne Muscular Dystrophy*

Michele A. Lloyd-Puryear [1], Annie Kennedy [1], R.R. Howell [2] and Jerry R. Mendell [3]

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Duchenne muscular dystrophy (DMD) is one of the ten most severe and common pediatric genetic diseases and affects an estimated 1 in every 3500–5000 male births. While DMD is a 100% fatal disease, in 2015 the therapeutic landscape changed and new treatments have been developed that target the different kinds of mutations causing DMD. Pending the launch of these new treatments, newborn screening of babies for DMD is critical. The current treatments are based on the various genetic mutations causing DMD and include:

TranslarnaTM: It is estimated that a nonsense mutation is the cause of DMD in approximately 13% of patients, or about 2000 patients in the USA/2500 in the EU. TranslarnaTM received marketing authorization in the European Union in August 2014 for the treatment of nonsense mutation DMD in ambulatory patients aged five years and older, representing the first-ever treatment approved for the underlying cause of the disease. The approval in the European Union is subject to PTC's obligation to provide data from the ongoing Phase 3 ACT DMD trial by the end of 2015.

Sarepta: Confirmatory trials for another promising therapeutic intervention began for the exon skipping therapy being led by Sarepta Therapeutics in the USA and an accelerated approval pathway for review commenced in 2015 which could benefit yet another 13% of the Duchenne population whose disease may be modified through a skipping of the targeted exon 51. After 3 years of eteplirsen treatment the six-minute walk distance was 151 m better than natural history controls and fewer treated DMD patients had lost ambulation.

KyndrisaTM: Biomarin began the regulatory process under a new drug application for their exon skipping therapy, which would potentially benefit the same subset of the Duchenne population.

This session will present a summary of the master plan for the development of our pilot for newborn screening in the U.S.A. and activities accomplished to date.

O18. Development of a New Bloodspot Screening Assay for Duchenne Muscular Dystrophy (Dmd)

Stuart Moat, Petra Feru, Teemu Korpimäki, Harri Hakala, Hanna Polari and Ian Weeks

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Background: Duchenne muscular dystrophy (DMD) is a progressive, lethal X-linked neuromuscular disorder with an estimated worldwide incidence of ~1:5000 male live births. The Wales newborn screening programme for DMD was operational for 21 years (1990–2011) and utilised a bloodspot creatine kinase (CK) enzyme activity assay. A total of 343,170 boys were screened and of the 145 cases with a raised CK, 56 were confirmed to have DMD. The Wales programme was terminated following the withdrawal of the external quality assurance scheme and due to a lack of sustainability of the reagents used. This programme was the second longest running in the World, but was the most comprehensive with respect to follow-up/outcome data. This long term study has so far identified 15 false negative cases. It is difficult to assess the false negative rates in other programmes as the vast majority were smaller and were operational for short periods or did not record such cases. The existing bloodspot CK assays are non-specific as total CK activity is measured. CK is an isoenzyme and it is the CK-MM isoform that is found predominantly in skeletal muscle and is elevated in boys with DMD. There is renewed interest in implementing screening for DMD as early intervention with steroids improves outcomes and the prospect of molecular therapies on the horizon.

Objectives: To develop a sensitive and specific immunoassay to detect CK-MM in bloodspots.

Results: CK-MM concentrations in bloodspot samples from DMD patients (1217–9917 ng/mL, n = 10) were higher than those observed in routine newborn bloodspots (29–461 ng/mL, n = 296). The CK-MM concentrations in DMD samples correlated with the measured CK activity. CK-MM concentrations in the normal population separated well from background concentrations. This assay has been adapted onto the PerkinElmer GSP analyser with inter-assay CVs < 10%.

Conclusion: CK-MM can be reliably detected in bloodspots. Adaption of this assay onto a commercial immunoassay-analyser would enable robust high-throughput screening for DMD.

O19. Recommendation of X-Linked Adrenoleukodystrophy Newborn Screening in The United States: The Roles of Evidence, Experts, and Advocacy

Jennifer Kwon

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In February 2016, X-linked adrenoleukodystrophy (X-ALD) was added to the United States' (US) Recommended Uniform Screening Panel (RUSP). The Secretary of Health and Human Services approved it based on the recommendation of the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) which reviewed the available evidence about X-ALD newborn screening (NBS), assessed its public health impact, and heard public commentary from advocates. While the process for adding conditions to the RUSP has been well documented and parallels other systematic national approaches to NBS, the approval of X-ALD in the US is worth examining, because X-ALD seems like an unusual choice for NBS: it has markedly heterogeneous clinical presentations requiring medical surveillance over decades to determine safe timing of treatments. The formal evidence review and the public assessment by the ACHDNC highlighted gaps in the evidence supporting X-ALD NBS and in the assessment of potential harms. This presentation will introduce the US process of X-ALD condition review with video and transcripts of the ACHDNC proceedings, and it will highlight the role of advocacy groups in influencing the perceptions of the ACHDNC. There will also be discussion of the clinical safeguards being proposed to address the harms that have been seen in the first year of X-ALD NBS in New York State, the only state that currently screens for X-ALD. The goal of this presentation is to illuminate the process in the US by which a new disorder is added

to the RUSP and to review the evidence for X-ALD NBS, both prior to its addition to the RUSP and currently, based on recent clinical experiences.

O20. The Co-Operative Development of Newborn Screening in Emerging Economies; a Workable Model of Collaboration

James Bonham

Children's Hospital, Clinical Chemistry, Sheffield, UK

Newborn screening began in the United States and Europe approximately fifty years ago with phenylketonuria. It has been a success story and has brought huge benefit to many families. In the US alone it has been suggested that it saves or improves the lives of more than 12,000 babies each year. It is therefore not surprising that, as the health services in developing economies grow and mature, that attention is becoming focussed upon realising these benefits for their populations. Internationally, the number of babies screened is in excess of 15 million pa but is set to rise to around 60 million pa over the coming 5 to 10 years.

In light of this rapid growth it is important that the experience gathered by countries with mature health care systems is harnessed to ensure that in developing economies newborn screening is offered as an integrated programme and not just a test.

Three centres in the UK, working with Public Health England, have developed a model of support to provide access to information technology, education and training and on-going clinical support for screen positive cases where this is not available locally.

We are currently developing these collaborations with centres in Bangladesh, Iraq, Iran, Pakistan and India. Local sustainability and an integrated ethical approach to the introduction of these services is key to their long term success. This presentation will explore the experience, dilemmas and solutions with some case examples.

O21. Validation of a Smartphone Tsh Immunoassay for Point-of-Care Newborn Thyroid Screening

Joel Ehrenkranz, Michael Kappy and Michael Levine

i-calQ, Medicine, Salt Lake City, UT, USA

Identification of newborns with congenital hypothyroidism is critically important, as delayed diagnosis and treatment can result in permanent and irreversible neurocognitive impairment. Nevertheless, only an estimated 1/3 of the worldwide birth population is screened. Conventional assays for TSH and/or T4 using dried blood spots (DBS) are affected by pre-analytic variables, especially hematocrit and ambient temperature and humidity, and require transport to a specialized resource-intensive laboratory.

To increase the availability and accessibility of newborn screening for congenital hypothyroidism and overcome the limitations of conventional DBS screening approaches, we have developed a point-of-care immunochromatographic TSH assay that uses 20 microliters of serum or plasma, delivers results in 15 min, and employs a smartphone or tablet for assay quantification and data archiving. This single-use, disposable noncompetitive assay format contains a mobile phase mouse monoclonal anti-beta TSH antibody covalently labeled with silica shelled gold nanoparticles and a goat polyclonal anti-beta TSH capture antibody bound to a nitrocellulose membrane. The assay is linear over the clinically relevant range of 1.0–80.0 mIU/L, has a coefficient of variation of 10%, and generates results that correlate with a third generation TSH chemiluminescent assay ($n = 256$; $r = 0.98$). In addition, the assay shows no cross reactivity with LH (45.3 IU/L), FSH (75.4 IU/L), or hCG (203,409 IU/L), does not have a high dose hook effect (TSH 530 mIU/L), and is not affected by serum contamination with bilirubin (34.5 mg/dL), triglycerides (3607 mg/dL), or hemoglobin (500 mg/dL).

The smartphone point-of-care TSH immunochromatographic assay can reliably measure TSH elevations found in primary congenital hypothyroidism. Bedside newborn screening for congenital

primary hypothyroidism using a disposable TSH immunoassay and smartphone provides results in minutes and is not subject to pre-analytic variability due to hematocrit or ambient temperature and humidity.

O22. Interim Results of a Pilot Newborn Screening Program in Nepal

Ralph Fingerhut and Arti S. Pandey

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During the last years, there were numerous countries that celebrated 50 years of newborn screening (NBS), and within the next years, more will follow. The International Society for Neonatal Screening (ISNS) will also celebrate at the conference in The Hague: "ISNS silver jubilee—25 years of sharing knowledge globally". And while Switzerland celebrated its 50 years NBS anniversary, the Kathmandu Medical College and Teaching Hospital and the Children's Hospital in Zurich started their collaboration with a pilot NBS program for Nepal. This program is supported by the Nepal Health Research Council (NHRC), and will comprise 5000 dried blood samples from Nepal in the first phase. We can present the interim results of the first year, from March 2015 to March 2016, of this pilot study. Until now 3786 samples have been tested. Within this cohort we could detect 1 case with congenital hypothyroidism and one case with cystic fibrosis. At the moment we are planning to extend the pilot to a total number of 10,000 samples, and explore the possibility further to transfer knowledge and methodology to Nepal.

O23. Genomic Newborn Screening: Public Health Policy Considerations and Recommendations

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VU University Medical Center, Section Community Genetics, Department of Clinical Genetics and EMGO Institute, Amsterdam, The Netherlands

Genomic technologies such as genome-wide sequencing, can identify genetic causes of rare paediatric diseases much more effectively than conventional clinical and laboratory methods. However, the nature of this technology and its potential to identify additional information about the child being tested raises a range of concerns, particularly when these technologies are applied in a public health setting, such as newborn screening (NBS).

The Global Alliance for Genomics and Health is an international collaboration of more than 370 healthcare, research, disease advocacy, life science, and information technology institutions formed to promote human health through sharing of genomic and clinical data (http://genomicsandhealth.org/). Within this remit, the Paediatric Task Team of the Global Alliance's Regulatory and Ethics Working Group was established to address issues of particular relevance to child health.

This Paediatric Task Team has developed the following recommendations for clinicians, clinical laboratory scientists, and policy makers regarding the use of genomic technologies for population-based newborn screening:

NBS by genomic methods should only be considered as an add-on to current screening programs, which should not be replaced unless equal or better sensitivity and specificity is shown. Equal availability and accessibility to every infant born in the jurisdiction should be guaranteed. Data sharing is needed for interpretation of variants. Publicly-funded universal newborn screening by genomic methods should be limited to diseases that can be effectively treated or prevented early in life. A program should guarantee treatment and follow up. We conclude we are not yet ready to implement sequencing large multigene panels in NBS.

O24. Incorporating Targeted Molecular Testing Methods in Newborn Screening

Jennifer Taylor, Scott Zimmerman and Shu Chaing

RTI International, Social, Statistical, & Environmental Sciences, Research Triangle Park, USA

Technological advances provide newborn screening with the capability to expand laboratory analysis for additional conditions using DNA testing. Although whole genome or whole exome sequencing could identify a number of specific disorders, sequencing also causes uncertainty in clinical outcome by identifying mutations of unknown significance. Targeted DNA testing is currently in use to supplement screening test and when biochemical testing is not available to identify and refer screen positive patients. In the United States, more than half of State-sponsored newborn screening programs are screening for severe combined immunodeficiency (SCID) using real-time PCR as the primary testing method. Real-time PCR can be used to multiplex other analytes in the same assay used to screen for other disorders, reducing the cost of adding conditions to the screening panel. Digital PCR is another platform with growing applications that have been shown to be useful in quantifying DNA and measuring copy number variations to detect conditions such as spinal muscular atrophy (SMA) and 22q11 deletion syndrome. Other platforms such as high resolution melting analysis and conventional PCR paired with capillary electrophoresis have been used in newborn screening pilots to identify SMA and fragile X syndrome (FXS). Molecular testing is the only option when screening for certain disorders such as SCID, SMA, or (FXS). This presentation will give an overview of targeted molecular testing methods, demonstrate how these methods can meet the needs of high-throughput newborn screening laboratories, and discuss the applicability of these methods to detect new disorders.

O25. *Parent Preferences for Return of Results from NGS: A Discrete Choice Experiment*

Don Bailey, Megan Lewis, Ryan Paquin and Holly Peay

RTI International, Early Education, Disability, and Health, Research Triangle Park, NC, USA

The perspectives of the public, expectant parents, and professionals inform researchers and policy makers about knowledge, acceptability, beliefs, perceived benefits, and concerns about newborn screening. The emergence of new technologies, such as next generation sequencing, and the pressure to expand screening to conditions with variable phenotypes and uncertain benefits, call for continued study of stakeholder perspectives. Although traditional surveys have provided much useful information, the complex decisions associated with new technologies suggest the need for methods capable of weighing the combination of factors that stakeholders must consider when assessing new screening options. This presentation describes a discrete choice experiment in which we presented a structured set of forced-choice questions with profiles describing conditions and outcomes associated with next generation sequencing. We demonstrate the utility of structured quantitative approaches such as discrete choice experiments in determining the information that is most important to families when they make decisions about newborn sequencing and the return of results.

O26. *Next Generation Sequencing in Newborn Screening for Cystic Fibrosis: Can We, and Should We?*

Mei Baker, Anne Atkins, Michael Rock and Philip Farrell

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Most programs use a method of analyzing a limited number of CFTR mutations (typically 23–40) following an immunoreactive trypsinogen (IRT) analysis on dried blood spot specimens (the 2-tier IRT/DNA algorithm). Infants with either 1 or 2 mutations detected are reported as screening positive and need further diagnostic evaluations by relatively difficult and expensive sweat testing procedures. This essential follow-up step requires the parents to travel to a CF center, which may be a long journey and, unfortunately, in 10%–20% of cases results in either an indeterminate or insufficient sweat test result. Moreover, greater than 90% of infants with one identified mutation have normal

sweat test results and are therefore CF heterozygote carrier infants who are categorized as screening false positives.

To reduce CF NBS false-positive results due to identification of carriers, we designed a study of a novel IRT/NGS (next generation sequencing) screening strategy for CF, taking advantage of pathogenicity information reported from the recently published CFTR2 project and ability of simultaneously detecting large number of mutations using NGS technology. Under the two-tier IRT/NGS strategy, we envision that eventually newborns with IRT below the cutoff value would be reported as CF screening normal (negative), and only newborns with two CFTR mutations would be reported as CF screening abnormal (positive).

We have successfully adopted an assay with the FDA-cleared Illumina MiSeqDx system using dried blood spot specimens that simultaneously detects 139 CF causing mutations, and further modify the mutation detection software component of the assay to increase the number of detected mutations up to 250. Our preliminary results indicates it is feasible to integrate NGS into a routine NBS program.

O27. Developement and Evaluation of a Newborn Screening Pipeline for Whole-Exome (WES) and Whole-Transcriptome (WTS) Sequencing from Dried Blood Spot Specimen—Focus on Leukocyte and Metabolic Disorders

Stephan Borte, Volker Menzel, Ulrich Sack, Michael Borte and Lennart Hammarström

ImmunoDeficiencyCenter Leipzig, Department of Pediatrics, Leipzig, Germany

Newborn screening programs (NBS) for severe primary immunodeficiencies (PID) have paved the way for high-throughput methods to extract and analyse nucleic acids from regular dried blood spot (DBS) specimen ("Guthrie cards") using sequence-specific methods such as quantitative PCR. However, existing PID-screening tests in the US and in a growing number of European countries solely rely on the detection of severe T and/or B cell lymphopenias (TREC and KREC assays) and thus do not detect severe functional immunodeficiencies (SCID) with preserved absolute numbers of T or B cells, nor phagocytic or complement deficiencies. As functional assays for lymphocyte proliferation and intracellular signaling cannot be performed on DBS specimen, massively parallel sequencing technology might provide sufficient bioinformatical information to rapidly delineate functional consequences of disease-causing sequence aberrations at the neonatal stage.

We have developed a high-throughput 96-well plate based method for extraction of DNA and RNA species to allow low-biased library construction for simultaneous whole-exome (WES) and whole-transcriptome (WTS) sequencing from regular neonatal DBS samples. This pipeline approach has been evaluated with regard to pre-analytical sampling, library-construction and sequencing, base-calling and targeted alignment algorithms and their applicability to a list a 220 PID entities, as well as 65 metabolic diseases and other disease entities enrolled in existing NBS programs. Furthermore, we will present on aspects of ethical considerations and cost-calculations for up-scaling.

Our insights from molecular NBS for PID indicate that peripheral blood sequencing reliably and efficiently reveals germline-sequence related disease entities of leukocyte disorders and selected metabolic diseases.

O28. Effect of Dried Bloodspot Quality on Newborn Screening Analyte Concentrations and Recommendations for Minimum Acceptance Criteria for Sample Analysis

Stuart Moat and Roanna George

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Background: The analysis of dried bloodspots has been used routinely for newborn screening world-wide for over forty years. The number of disorders screened for has expanded significantly in recent years. The quality of the bloodspots is assessed subjectively by visual inspection in the screening laboratory, and repeat samples are requested on those deemed unsuitable for analysis

However, the rejection of samples is not standardized, since no specific guidance exists to define the minimum quality acceptance criteria. The lack of consensus results in wide variation in practice, with different laboratories accepting or rejecting samples of differing quality, leading to difficulty in comparing avoidable repeat rates. A major concern is that there is a lack of scientific evidence to determine minimum acceptance criteria for bloodspot sample analysis.

Methods: Blood pools were spiked with phenylalanine, tyrosine, leucine, methionine, TSH, IRT, C8, C10, C5DC C5 at analytical cut-off concentrations used in the UK Newborn Screening protocols. The effect of sample volume, punch location and sample quality were evaluated.

Results: Smaller bloodspots produced significantly lower results (15%–25% for 10 µL vs. 50 µL sample size) for all analytes at all concentrations measured ($p < 0.001$). Results obtained from a peripheral punch are higher than those from a central punch for most analytes measured. Compression of bloodspots produce significantly lower results (15%–45%) for all analytes ($p < 0.001$).

Conclusions: Results from this study have shown that all spots containing <10 µL (bloodspot diameter <6–7 mm) of blood should be rejected, it is recommended that the initial punches taken for each assay should be from a peripheral location. All samples showing evidence of compression should be rejected as there is a significant risk of producing false negative results. These results have been used to underpin UK Bloodspot Quality Guidelines for Screening Laboratories which aim to improve patient outcomes.

O29. The Benefits and Challenges of Introducing the Newborn Blood Spot Failsafe Solution in England

Julie Wilcox

Public Health England, NHS Newborn Screening Programmes, London, UK

Although all babies born in England are entitled to newborn blood spot (NBS) screening, evidence shows that some are at risk of missed or delayed screening, particularly those moving between locations or if the screening card does not reach the laboratory on time.

As well as the potential harm to babies from screening delay, the cost of extra treatment, care and litigation is significant. Failures in the NBS screening pathway may also indicate wider failures in postnatal care.

The Newborn Blood Spot Failsafe Solution (NBSFS) is a web-based product which addresses the significant challenges of identifying unscreened babies. The NBSFS receives notification of every birth in England and matches it to every NBS sample received by the 13 newborn screening laboratories. Potentially unscreened babies are identified by the local maternity service who can then follow up. Because the data-base is national, babies moving between areas can be monitored. Clinical audit demonstrates that NBSFS is effectively detecting missed and delayed cases, and driving improvements NBS screening.

NBSFS has been implemented in all English maternity units and could be used by other screening professionals, such as health visitors and paediatric nurses. NBSFS can also be used to automate other parts of the screening process, such as producing reports and messaging other screening agencies, further improving quality. Other health-care systems could use a similar model.

This presentation will introduce the NBSFS, demonstrate its benefits, identify the challenges to implementation, and outline future developments.

O30. Expansion of a National Newborn Screening Quality Assurance Program—Technical Assistance From The CDC's Newborn Screening Quality Assurance Program

Joanne Mei, LiXia Li, Charity Jomento, Maria Truda Escoreal, Zobel Sherri, Irene Williams, Victor R. De Jesús, Carmencita David Padilla and Carla Cuthbert

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Background: The Philippines newborn screening system offers testing for Congenital Hypothyroidism, Congenital Adrenal Hyperplasia, Galactosemia, Phenylketonuria, Glucose-6-Phosphate-Dehydrogenase Deficiency, and Maple Syrup Urine Disease. They recently implemented expanded screening using tandem mass spectrometry (MS/MS) and screening for hemoglobinopathies, Cystic Fibrosis, and Biotinidase Deficiency. They also added new testing laboratories and regional comprehensive follow-up/treatment centers.

Methods: In 2015, the Newborn Screening Reference Center at the National Institutes of Health, University of the Philippines, Manila, requested technical assistance from the Newborn Screening Quality Assurance Program (NSQAP) at CDC. NSQAP hosted two Filipino scientists who received hands-on training on: how to prepare human blood products to produce dried blood spot quality control and proficiency testing materials; MS/MS using non-derivatized methods; fluorescence assays for enzymes; automated time-resolved fluorescence assays, and hemoglobinopathy testing. They also spent time with NSQAP data management staff to observe critical administrative functions for the operation of the program.

Results and Conclusions: The Philippines has a long history of supporting quality laboratory testing for newborn screening. It was an early international participant in NSQAP and it built a national QA system based on the CDC program. To ensure that a high degree of quality was maintained for expanded testing, CDC transferred technology to the Philippines so that it can provide QA materials and oversight for more national newborn screening laboratories. The partnership provided a model for how to grow a national newborn screening system through governmental collaboration.

O31. *Upcoming Nationwide CF Implementation in Germany—Chances and Potential Pitfall*

Theresa Winter, Cornelia Müller, Sebastian Schmidt and Matthias Nauck

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Objectives: In August 2015, the G-BA voted for the nationwide implementation of the Cystic fibrosis newborn screening (CF-NBS) in Germany. Until its final realization in 2016, Mecklenburg-Vorpommern (MV) is still the only German state, with an integrated CF-NBS for all newborn free of charge in due course of an EU founded Interreg IVa project.

The experience, gained since October 2012 is a valuable pool of information concerning the optimization of screening processes in accordance to the proposed CF-NBS screening protocol and analytical drawbacks.

Methods: The nationwide proposed CF-NBS protocol is three staged. The first two steps are conventional laboratory analysis of (1) immunoreactive trypsinogen (IRT) and of (2) pancreatitis associated protein (PAP). As a third step, a molecular genetic analysis will be conducted. CF-NBS will be positive in case of an elevated IRT > 99.9th percentile or with at least one mutated CFTR-gene.

In contrast, a two-step strategy was used since October 2012 in MV- including the analysis of IRT and PAP.

Results: Despite the missing molecular analysis, a profound data base with more than 55.000 screened newborns was established. The acceptance of the CF-NBS reached soon after its introduction more the 95%. The mean time between positive CF-NBS was significantly reduced to 33 days in 2015- ranging now in European cystic fibrosis society standards of care recommendations. Furthermore, the applied cut-offs for IRT and PAP detected all 7 positive CF-NBS newborns.

Conclusion: The challenges for the nationwide implementation are nevertheless divers. Challenging will be the inclusion of the third analytical step, the resulting tight time schedule until the sweat test and the safe use of the altered IRT cut-off- in order to reduce the false positives. Furthermore, reimbursement concerning the sweat tests, the additional laboratory analytic and the tracking are not solved yet.

O32. Newborn Screening for Haemoglobinopathies—A Cohort Study Evaluating the Completeness and Early Outcomes of the Newborn Sickle Cell Screening Programme in England 2010 to 2014

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Since 2005 all infants born in England are offered sickle cell disease screening as part of the newborn blood spot screening programme. An evaluation of all cases notified to the new-born outcomes project between September 2010 and August 2014 has been established to determine completeness of coverage, timeliness of entry into care, and penicillin prophylaxis and to ascertain all causes of mortality as per the standards for the new-born screening programme https://www.gov.uk/government/publications/standards-for-sickle-cell-and-thalassaemia-screening. 1398 babies born between September 2010 and August 2014 in England with a screening result of sickle cell disease or beta thalassaemia were identified by the designated new-born screening laboratories. After excluding those born abroad, opt outs and insignificant cases and those who left England shortly after birth there were 1087 case of sickle cell disease followed up. Children eligible for penicillin (excluding those with the double heterozygote HbS/HPFH) had all been prescribed although only 88% by 3 months of age. Similarly only 85% of children had been seen in clinic by 3 months of age. There were 9 deaths in the sickle cell cohort, 3 due to Streptococcal septicaemia and acute splenic sequestration that were related to their condition. The other 6 deaths were not related to date there have been no cases of false negatives i.e. babies missed who have subsequently turned out to have sickle cell disease. The new-born screening programme has been well accepted and there was only one case of opt out of screening. One unit requested parental consent despite the national permission for named data without consent and this led to 5 families opting out of registration. Timeliness of entry into care and penicillin prophylaxis has improved over the 4 years of the study. Deaths still occur in the first 5 years of life from sickle cell complications despite penicillin prophylaxis and conjugate pneumococcal immunisation and families and professionals need to be aware of this and remain vigilant for invasive pneumococcal sepsis in these children.

O33. Clinical Presentation, Gene Analysis and Outcomes in Young Patients with Early-Treated Combined Methylmalonic Acidemia and Homocysteinemia (CBLC Type) in Shandong Province, China

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Objectives: To estimate the incidence of MMA on newborn screening in Shandong province from May 2011 to May 2014 and summarize the clinical presentation, biochemical features, mutation analysis, and treatment regime of early-treated patients with cblC disease.

Methods: Between May 2011 and May 2014, 35,291 newborns were screened for MMA in Shandong province. The levels of C3, C3/C2, methionine and tHcy were measured. Most patients received treatment with intramuscular hydroxocobalamin after diagnosis. Metabolic parameters, clinical presentation and mental development were followed up.

Results: Nine patients were identified among 35,291 by newborn screening, giving an estimated incidence of 1:3920 live births for MMA, and all were classified as cblC disease. Among them, five patients received treatment and two patients did not receive any treatment. One patient died of metabolic crises triggered by infection at the age of 38 days. Seven different mutations (c.609G>A, c.455_457delCCC, c.394C>T, c.445_446insA, c.658_660delAAG, c.452A>G and IVS1+1G>A) were detected. The mutations (c.455_457delCCC and IVS1+1G>A) are novel. Five patients who received treatment had favorable metabolic response. We obtained 7 records of DQ assessment. The five patients who received treatment presented with developmental delay and obvious neurological manifestations.

In two patients who did not receive any treatment, case 8 presented with severe mental retardation and developmental delay, while case 9 had nearly normal DQ values at the age of 11/12 years.

Conclusion: Our study characterized variable phenotypes of neurodevelopment in early-treated cblC patients diagnosed on newborn screening. The long-term outcomes of cblC disease are unsatisfactory in spite of conventional treatment and improvement of biochemical abnormalities. Although the number of patients is too small, the information provided in this work is of value in highlighting possible genotype-phenotype correlation that influences outcomes in cblC disease by future studies.

O34. Morbidity and Mortality Among Exclusively Breastfed Neonates with Medium Chain Acyl-Coa Dehydrogenase Deficiency

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Background: Since widespread implementation of universal newborn screening (NBS) using tandem mass spectrometry, morbidity and mortality from MCAD has decreased drastically. However, it is known that some infants become ill prior to NBS results being available. Previous research reported that among MCAD patients born in the early 2000's, 4%–5% were symptomatic before their NBS resulted. Among this population, exclusive breastfeeding was associated with symptomatic presentations and higher octanoylcarnitine levels. Since this time rates of exclusive breastfeeding have increased nationwide. In this study we set out to quantify the current risk of early clinical decompensation in neonates with MCAD. We also worked to identify factors associated with poor outcomes prior to return of NBS results.

Methods: This is a retrospective analysis of all neonates referred to our center and confirmed to have MCAD after an abnormal newborn screen between January 1st 2010 and August 1st 2015.

Results: 46 infants were diagnosed with MCAD during the study period. 11 of 46 (23.9%) were symptomatic prior to or at the time of the abnormal NBS report. Of these patients 4 presented with death or cardiac arrest, and 7 had hypoglycemia and/or lethargy. 100% of symptomatic patients were exclusively breastfed, while only 40.6% of asymptomatic patients were exclusively breastfed ($p = 0.0008$). During the study period, there was an upward trend in breastfeeding rates among patients without a known family history of MCAD. In 2010-2011 45.5% of patients were exclusively breastfed; this increased to 64.7% in 2012–2013, and 87.5% in 2014–2015. Over this same time period rates of decompensation prior to NBS results significantly increased from 9.09% in 2010–2011, to 23.5% in 2012–2013, and 75% in 2014–2015 ($p = 0.003$).

Conclusions: Exclusively breastfed neonates with MCAD are at risk for early metabolic decompensation. As breastfeeding rates rise, close management of feeding difficulties is essential for all neonates awaiting NBS results.

O35. mTFP Deficiency Missed in Newborn Screening

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Newborn screening for defects in long-chain fatty acid oxidation is a particular challenge as acylcarnitine profiles may normalise intermittently despite a clinically relevant enzyme deficiency. We report on a girl with 2 acyl carnitine profiles suggestive first of MAD, then of mTFP, that was finally judged as unaffected following a normal 3rd profile.

mTFP-deficiency was diagnosed at the age of 4 years after repeated episodes of prolonged recovery from minor infections and chronic exercise intolerance. The acylcarnitine profile turned

pathological during a febrile episode revealing the profile of mTFP/LCHAD again. Molecular genetic analysis revealed compound heterozygosity for a known and a so far unknown mutation(s).

Conclusion: It is of utmost importance to involve a specialist metabolic unit early in the work up in patients with a suspicious screening results.

O36. Newborn Screening Continuity Clinics—Improving Long-Term Follow-Up Care and Strengthening the Infrastructure for the Philippine Expanded Newborn Screening

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The increasing national coverage of the newborn screening (NBS) and the expansion of the program screening for 28 disorders from the basic six-test panel were foreseen to identify more and more babies with inherited metabolic conditions, endocrine disorders and hemoglobin disorders. Recognizing that these babies will be needing subspecialist care which is limited to the tertiary medical centers usually located in the big cities, the National Comprehensive Newborn Screening System sought the establishment of continuity clinics in each region of the Philippine archipelago. These clinics are expected to take on the long term and follow-up care of affected babies. With this specific task, these clinics aim to improve the long-term outcome of these babies through regular monitoring and timely medical management and referral to subspecialists—metabolic physicians, pediatric endocrinologists, hematologists, pulmonologists, clinical geneticists among others. Beyond screening, the establishment of these clinics brings closer to the affected babies and families the needed medical care to help ensure that these affected individuals live healthy and productive lives.

Over a year since their establishment, data on follow-up and monitoring, as well as challenges encountered will be presented.

O37. Neuropsychological Outcomes in Children with Subclinical Congenital Hypothyroidism

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Background: Untreated severe congenital hypothyroidism (CHT) results in intellectual retardation, but there is debate whether mild abnormalities in thyroid function pose any risk to childhood development. In New Zealand newborn screening TSH levels <15 mIU/L blood (termed subclinical congenital hypothyroidism; SCHT) are not investigated further. Whilst other countries use lower TSH cut-offs, it is unclear whether SCHT has a long-term impact on neurocognitive development. We hypothesised that children with SCHT at birth would display no adverse cognitive outcomes.

Methods: Healthy subjects aged 6–11 years with SCHT at birth ($n = 53$, screening TSH 9–14 mIU/L blood) were identified from the national newborn screening database. Sibling controls aged 6-16 years were also recruited ($n = 50$). Thyroid function was assessed on SCHT participants and all participants underwent a comprehensive cognitive assessment (primary outcome IQ as measured by WISC-IV), with results compared for SCHT and control groups.

Results: Mid-childhood TSH levels had decreased to the normal range in all SCHT subjects (mean TSH 2.31 mIU/L). There were no group differences in full-scale or sub-component IQ scores between SCHT and sibling controls (full scale IQ 110 ± 1.7 vs. 109 ± 2.1 SCHT, $p = 0.63$). Increasing newborn TSH within the SCHT group was associated with lower IQ scores; full-scale IQ ($\beta = -2.89$, $p = 0.002$), verbal comprehension ($\beta = -4.15$, $p = 0.002$).

Discussion: Children with untreated newborn SCHT performed as well as sibling controls on neurocognitive tests. However, mildly increased newborn TSH was associated with reduced IQ. We speculate that a common environmental factor may be responsible for both the rise in TSH and lower mid-childhood IQ scores.

O38. Improvement of the Validity of the Dutch Combined Irt/Pap/Innolipa/Ega Newborn Screening Program for CF by Re-Evaluating Mutation Classification and Optimizing Cut-off Values

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Since May 2011, newborn screening for Cystic Fibrosis (CF) is part of the Dutch program. The screening consists of a 4-step strategy (Immunotrypsinogen (IRT)/Pancreatitis-associated Protein (PAP)/Line probe assay (InnoLiPA) for 35 CFTR mutations/extended gene analysis (EGA) as reported before (Thorax 2012; 67, 289–295)). Newborns with at least one mutation are referred for a sweat test. Between 1 May 2011 and 30 June 2014, 12 newborns were missed by screening, resulting in a sensitivity of 91 (CI 83–95)%, a specificity of 99.99% and a positive predictive value (PPV) of 65%.

Objective Can the performance of the screening program be improved by alternative cut-off values (COV) for IRT and PAP, combined with re-evaluated classification of mutations?

Methods The InnoLiPA panel was re-evaluated in order to reduce the number of referred newborns with inconclusive diagnosis for CF (CFSPID). Using the re-assigned panel, we calculated sensitivity, specificity, PPV and costs of 11 scenarios with different COV.

Results after changing the classification of the R117H-7T/9T mutations from "inconclusive" to "no clinical relevance", six scenarios showed a sensitivity ⩾95%. A scenario where DNA analysis are performed if IRT ⩾ 100 µg/L and PAP ⩾ 1.2 µg/L, or IRT ⩾ 60 µg/L and PAP ⩾ 3.0 µg/L, or IRT ⩾ 124 µg/L blood regardless of PAP, was considered the best option, with a sensitivity of 95 (CI 88–98)%, specificity of 99.99% and PPV of 60%. Costs per test will rise from € 3.25 to € 3.42. Annually, the number of CFSPID will decrease from 7 to 1, but the number of referred carriers will slightly increase from 10 to 14.

Conclusions Sensitivity of the Dutch CF screening program can increase with acceptable specificity, PPV, costs and number of referred carriers.

4. Poster Presentations

P01. Evaluation of 12 Years Neonatal Screening for Congenital Adrenal Hyperplasia in The Netherlands

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Introduction: Screening for congenital adrenal hyperplasia (CAH) was implemented in the Netherlands in 2002. Aim of the Dutch screening is the early detection of children with the classic salt-wasting (SW) form of CAH, a 21α hydroxylase deficiency, resulting in a lack of cortisol and mineralocorticoid synthesis. We evaluated prevalence, validity and percentage of patients detected by screening in relation to clinical detection.

Methods: The heel prick is performed between day 4 and 7 after birth. 17 OHP is measured with the AutoDELFIA method. Cut off values are based on gestational age and weight. In case of unequivocal screening results a second sample is requested.

Results: In total 486 neonates had a positive screening result. 114 patients (positive predictive value 23%) were diagnosed with SW CAH (64% boys) and 18 with SV CAH. 29% SW CAH patients (33/114) were detected on clinical grounds. 70% of patients detected on clinical grounds were girls (*n* = 23); 20 with ambiguous genitalia, 3 were diagnosed prenatally, 10 boys were also found before screening. 106 children with a positive screening result were born prematurely of which 2 patients had

SW CAH. PPV in this subgroup was 1.9%. No patients with SW CAH were missed (sensitivity 100%). Specificity was 99.98%. Prevalence of SW CAH was 1:20,000 (0.51 per 10.000).

Conclusion: Most patients were detected with the screening program. A striking result was a skewed sex ratio. Although sensitivity of the program is optimal, specificity is good and positive predictive value is acceptable, there are still many premature children with false positive results. We are looking for ways to improve this.

P02. The Prevalence, Clinical and Molecular Characteristics of Congenital Hypothyroidism Caused by duox2 Mutations: A Population-Based Cohort Study in Guangzhou

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Context: Thyroid dyshormonogenesis (DH) have recently been reported to be more frequently associated with mutations in the dual oxidase 2 (DUOX2) gene.

Objective: This study aimed to investigate the prevalence, clinical and molecular characteristics of congenital hypothyroidism (CH) caused by DUOX2 mutations in Guangzhou.

Patients and Methods: A population-based cohort of 156 patients with CH was recruited based on neonatal screening among 433,578 newborns born in Guangzhou from 2011 to 2012. Genetic analysis of DUOX2 was performed in 96 patients with DH by PCR-amplified direct sequencing method.

Results: Apart from 2 cases without ultrasonographic data, 118 (76.6%) of the 156 patients were classified as DH and 36 (23.4%) as thyroid dysgenesis (TD) according to thyroid ultrasound at diagnosis. Genetic analysis of DUOX2 revealed 26 different mutations in 62 unrelated individuals (62/96, 64.6%), including 17 novel mutations that were absent in the 100 normal control alleles and predicted to be pathogenic by SIFT and PolyPhen-2. The p.K530X mutation was the most frequent mutation. Ninety-two percent of mutant alleles occurred in exons 5, 6, 9, 14, 17, 20, 25, 27, 28. There were no significant differences in phenotypes between biallelic and monoallelic mutations cases or between with-DUOX2 and non-DUOX2 mutations cases. Most patients (71.7%) with DUOX2 mutations were transient CH.

Conclusions: DH accounted for the majority of CH in this cohort, with the prevalence of DH caused by DUOX2 mutations estimated at 1:5,617. The p.K530X mutation may be a mutational hotpot in the Chinese population. We did not find association between DUOX2 genotypes and clinical phenotypes.

P03. Increasing Coverage with Neonatal Thyroid Screening in the Republic of Macedonia, during 2002–2015

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Neonatal thyroid screening allows early diagnosis and treatment of congenital hypothyroidism and it is considered as appropriate approach in prevention of long term morbidity. In Macedonia, newborn thyroid screening program was established as nationwide in 2007, after five years as a pilot study.

Newborns from all public and private birth centers in the country are screened in the centralized laboratory located at University Pediatric Clinic in the capital of Macedonia, between 2002 and 2015. Thyroid-stimulating hormone (TSH) levels were analyzed from dry blood spots on filter paper using the DELFIA method.

During the study period, out of 259,522 live births 251,008 have been screened. The coverage of the screened newborns was 96.7% average, ranging from 88.3% in 2003 to 98.3% in 2015. There

is continuously increasing of coverage in the last five years (96.7% in 2011, 97.3% in 2012, 97.8% in 2013, 98.5% in 2014 and 98.6% in 2015) as a result of improved education and training of the personnel in the births centers across the country. We found coverage above 99% in 9.38% of the birth centers, above 98% in 28.13%, above 97% in 65.63% and coverage above 92% in 86.24% of the birth centers.

Neonatal thyroid screening program has changed the outcome of congenital hypothyroidism in the country. It has been satisfactory implanted in Macedonia with noticeably increased coverage over the last years. For improving the efficiency of the program an additional education in birth centers with lower coverage as well as covering the babies born at home are necessary.

Keywords: Coverage, Newborn screening, Thyroid-stimulating hormone

P04. Impact of Lower Screening TSH Cutoff Level on the Overall Incidence of Congenital Hypothyroidism

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The incidence of congenital hypothyroidism (CH) has increased in several countries. Lower cutoff levels in screening programs have led to an increase in the proportion of detected cases with transient hypothyroidism, leading to increase of the overall incidence of primary CH.

Total of 251,008 (96.72%) neonates were screened for thyroid-stimulating hormone (TSH) level in dried blood spot specimens taken 48 h after birth, between 2002 and 2015, using DELFIA method, a TSH value of 15 mU/L was used as the cutoff point up to 2010 and 10 mU/L thereafter.

Primary congenital hypothyroidism was detected in 127 newborns with overall incidence of 1/1976, and female to male ratio 1.35:1. Among neonates with primary CH, 103 (81.1%) had permanent CH with female predominance (female to male ratio 1.71:1) and 24 (18.9%) had transient CH with male predominance (female to male ratio 1:2). Interestingly, the incidence of primary CH was significantly increased from 1/2489 up to 2010 to 1/1585 thereafter, probably due to the lowering of the TSH cutoff level. The incidences of transient CH of 1/45,625 up to 2010, and more than eight times higher incidence, 1/5435, thereafter, were detected. Opposite, the incidences of permanent CH, before (1/2632) and after (1/2238) changing the TSH cutoff level, were slightly different.

Our findings support the impact of lower TSH cutoff on the increasing incidence of primary CH, especially of the transient CH, in the country. Further analysis is necessary to identify the other factors associated with increasing incidence of permanent as well as transient CH in Macedonia.

Keywords: Congenital hypothyroidism, Incidence, Neonatal screening, Thyroid-stimulating hormone, Cutoff level

P05. Variation of the Incidence of Congenital Hypothyroidism in Different Regions of Macedonia–Fourteen Years Newborn Thyroid Screening

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Neonatal thyroid screening program allows early effective diagnosis and treatment of congenital hypothyroidism (CH), the most common preventable cause for intellectual disability in children. The incidence of CH by regions in the Republic of Macedonia has not been separately evaluated before as well as its correlation with the percent of neonates with TSH concentration above 5 mIU/L, as an indicator for the assessment of iodine deficiency in the population.

Newborn thyroid screening (*n* = 251,008) has been performed in all eight regions of the country, by measuring thyroid-stimulating hormone (TSH) from blood spots on filter paper (Whatman 903), sampled 48 h after birth, using DELFIA assay, between 2002 and 2015.

We detected overall incidence of congenital hypothyroidism of 1/1976 in the country with different regional distribution. The incidences of CH by regions were following: Eastern Region 1/4202, Northeastern Region 1/1459, Pelagonia Region 1/1627, Polog 1/1444, Skopje Region 1/2430, Southwestern Region 1/3226, Southeastern Region 1/1843 and Vardar Region 1/970. Interestingly, in the Vardar Region with the highest incidence of CH we found 4.44% newborns with TSH concentration above 5 mIU/L compared to the Eastern Region with the lowest incidence of CH and 1.66% newborns with TSH> 5 mIU/L.

The incidence of CH significantly varies among the regions of the country compared to the incidence in the whole country. Assessment of the iodine status in the regions with high incidence of CH, especially in the Vardar Region, is necessary.

Keywords: Congenital hypothyroidism; Newborn screening; Thyroid-stimulating hormone; Region; Iodine intake

P06. *Rapid Test for the Detection of Thyroid-Stimulating Hormone (hTSH) in Newborns Associated with Congenital Hypothyroidism from a Blood Sample*

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Congenital hypothyroidism (CH) is the most serious cause for elevated thyroid-stimulating hormone (thyrotropin, hTSH) in a newborn. CH occurs when infants are unable to produce sufficient amounts of thyroid hormone (thyroxine, T4), which is necessary for normal metabolism, growth and brain development. In case CH is unrecognized, it can lead to developmental delay and mental retardation. Newborn screening and thyroid therapy started within two weeks after birth improve intellectual outcomes greatly. If hTSH is elevated, the possibility of CH needs to be assessed by repeated hTSH and free T4 measurements. Point of Birth TSH Test is a rapid test for qualitative determination of elevated hTSH in blood specimens as a primary screening of babies for CH. Point of Birth TSH Test is intended for initial screening of newborns after 48 h of age. The sensitivity of the test is $\geqslant$ 15 mIU/l hTSH.The test requires only 1 drop of blood from the pricked heel of the baby and it can be performed in 5–10 min. This new rapid test brings the newborn screening affordably to emerging markets without the need for centralized laboratory systems.

P07. *Establishing Newborn Screening Program for Congenital Hypothyroidism in City of Lahore Pakistan*

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In Pakistan the reported incidence of congenital hypothyroidism in newborns is 1:1000 and is even high in iodine deficient areas.

A decade ago, International Atomic Energy Agency (IAEA) under Human Health Program have addressed this problem by organizing training courses, workshops and support for establishing National reagent laboratory with the availability of radioimmunoassay technology in INMOL Lahore. The efforts started by collaborating with one private hospital in initial years and now blood spot screening is established in three private hospitals and 80% newborns are being screened for CHT, 24 h after birth.

To establish this program in Government Hospitals and at community level PHRC Centre NHRC based at SZMC started screening Congenital Hypothyroidism in Shaikh Zayed Hospital with in-house

Enzyme Immunoassay for blood spot TSH. The aim for both INMOL and NHRC, every newborn in the city of Lahore should be screened as pilot and same should be replicated in other areas of province of the Punjab followed by other provinces of Pakistan.

In 2014 PHRC Centre NHRC has involved Department of Health, Govt. of the Punjab and has collaborated with Institute of Education, University College London under HEC & BC grants, to establish and implement, newborn bloo132d spot screening program in Govt. hospitals and maternity units up to district level in Lahore as pilot.

The efforts done to adapt UK newborn screen program for CHT and the outcome achieved will be presented in the conference.

P08. Getting Research into Practice: Establishment of National Congenital Hypothyroidism Screening Program in Sri Lanka

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Despite attempts over time to begin organized newborn screening in Sri Lanka its implementation has been slow, mainly due to economic factors. The Ministry of Health has agreed to implement neonatal screening for congenital hypothyroidism (NSCH) in 2010 following several studies done by the Nuclear Medicine Unit (NMU). The established program has been able to achieve 96% coverage among live births within a year of implementation and maintained it so far. In this program there was a marked decrease in false positive rate (0.70% to 0.05% in 2011 and 2015 respectively) among screened infants. The incidence of CH was 1:1770 in 2011 to 1:1417 in 2015. On average the NMU was able to detect a true positive baby out of 14 false-positives in 2011 but in 2015 one out of two screening positive babies was confirmed as having CH. The implementation of this program was met with considerable challenges due to paucity of resources. However, with dedicated staff and limited facilities the NMU has been able to convince the success of the progress so that a national NSCH was established in January 2016. Efficient data collection and communication between parents and the medical service providers were improved with help of modern technologies. Web based tool was developed for data collection; internet short message system was established for communication between the parents and medical personnel. All these improvements for the screening program are working towards the goal of achieving nationwide coverage.

P09. Congenital Hypothyroidism or Mislabelled Sample—A Case for Repeat Assessments

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Introduction: In New Zealand, screening for congenital hypothyroidism (CH) is based on detection of elevated thyroid stimulating hormone (TSH). The laboratory performs urgent repeat tests in duplicate on samples where whole blood TSH $\geqslant$ 30 mIU/L, and these infants are subsequently referred to a Paediatric Endocrinologist. The positive predictive value of CH in directly referred babies (TSH $\geqslant$ 30 mIU/L, blood) is close to 100%.

Case Description: The TSH screen on a day 2 sample was elevated at 88 mIU/L (blood), confirmed on repeat assay. The infant (Baby J) was reviewed by a Paediatric Endocrinologist on day 5 and, surprisingly, found to have normal diagnostic thyroid function tests (serum TSH 8.7 mIU/L and free T4 22 pmol/L). There was no history of infant or maternal iodine exposure. The clinical director of the neonatology unit where the sample had been taken was notified and the screening laboratory performed urgent repeat tests on all samples received the same day as Baby J. Three residual samples that had been taken from Baby J for bilirubin measurements on days 4–5 were analysed; serum TSH ranged from 9–28 mIU/L. DNA testing on the original dried blood spot confirmed that it came from

baby J. Repeat thyroid function tests on day 17 were consistent with severe hypothyroidism (serum TSH 240 mIU/L, free T4 2 nmol/L, eutopic gland on scintiscan) and treatment commenced.

Discussion: This is an unusual case of anomalous thyroid function on newborn screen and follow-up tests. Given the possibility of a mislabelled specimen and second infant with unrecognised severe hypothyroidism, it was essential to rapidly confirm the identity of the infant sampled. Although it is not clear why the infant had a period of "normal" thyroid function tests, repeat assessments confirmed the presence of severe congenital hypothyroidism.

P10. Genetic Analysis for 36 Phenylalanine Hydroxylase Deficiency in Fujian Area

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Objective: To study the characteristics of Phenylalanine Hydroxylase (PAH) mutations in patients with PAH deficiency in Fujiannese populaton.

Methods: Peripheral blood samples of 36 patients and their parents with classical type PKU were collected and genomic DNA was extracted. PCR sequencing was carried out to identify the origins of mutations.

Results: 22 different mutations were identified in sixty-three of the 72 chromosomes. The most common mutations, R241C, R408Q and Ex6-96A>G, account for 15.9%, 12.7% and 11.1%, of the mutant alleles, respectively. The c.970-116_970-111delTAGACA and c27704-27705dupTGAC mutations are first reported in this study. R241C is associated with 28% mild hyperphenylalaninemia (MHP) and R408Q is associated with 25% classical PKU.

Conclusion: There is a specific spectrum of PAH gene mutation in Fujian area. R241C, R408Q and Ex6-96A>G are the most three common mutations.

P11. Screening of PKU in Romania—From Pilot Project to National Program

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Background: The screening of PKU began in Romania in 1979 as a pilot project developed in 2 counties. Since 2002 the screening program extended to 5 counties. Beginning with 2005 the PKU pacients receive medical food supplements granted by government. In all this time the number of counties extended continuously. Since 2011 the screening was extended at national level all the 42 counties being involved.

Method: The blood samples at screening are collected on dry spot. The laboratory methods used in screening started with Guthrie test followed by the fluorimetric method. Now in 2016 we are using mass spectrometrie tandem MS/MS. Beginning with 2011 quantitative determination of plasmatic Phe, through HPLC is used for confirmation of diagnostic and monitoring of patients.

Evolution: Between 2002 and 2015 the percentage of newborns screened for PKU increased from 24% to 94%. The number of diagnosed and treated children increased continuously from 24 to 122 children presently.

Since 2014 the dietary treatment has been associated with pharmacological treatment with sapropterine to the sapropterine loading test responsive PKU patients.

Conclusion: The extension of the newborn screening at national level, as well as the improvement of laboratory testing methods and treatment give the PKU patients a chance to have a normal life and to be integrated into the community.

P12. Classical PKU with Unusual Neonatal Presentation

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Newborn screening (NBS) for phenylketonuria (PKU) started in Switzerland in 1965. Since 2005, NBS for PKU using tandem mass spectrometry is performed at the University Children's Hospital in Zurich. Phenylalanine (Phe) and the phenylalanine/tyrosine (Phe/Tyr) ratio are sensitive and specific tests to detect phenylketonuria (PKU). The main clinical features of untreated PKU are developmental delay and intellectual disability, which become evident from the first weeks of life.

We describe a boy born at term to non-consanguineous Swiss parents with tetrahydrobiopterine (BH4) sensitive PKU with unusual neonatal presentation. The child presented with floppiness, irritability, recurrent bilious vomiting and failure to pass meconium until 32 h after birth, resulting in the clinical suspicion of an intoxication type metabolic disease such as maple sirup urine disease (MSUD) rather than PKU. Ketonuria or hyperammonaemia were not observed.

Newborn screening performed on a dried blood sample from the 4th day of life revealed elevated Phe of 650 µmol/L, low Tyr of 30 µmol/L, and a Phe/Tyr ratio of 22. However, slightly elevated branched-chain amino acids (leucine/isoleucine 306 µmol/L; valine 299 µmol/L) initially supported the clinical suspicion of MSUD, but alloisoleucine was not detectable. Morbus Hirschsprung was suspected due to dilated intestinal loops and lack of intestinal gas in the anorectal region. On day 8 branched-chain amino acids had normalised; A BH4-test resulted in a significant decrease of Phe from 1011 to 437 µmol/L within 24 h with normal pterin measured in urine; supporting the diagnosis of BH4 sensitive PKU. Dietary restriction of Phe was initiated immediately. However, oral feeding turned out difficult due to the intestinal motility disorder.

P13. Development of a gc-ms Analytical Method to Detect Organic Acidemia from Paediatric Urine

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Congenital disorders like Inborn Errors of Metabolism (IEM), leads to disability and death of an infant. Some of these disorders have safe and effective management, and if treated early can prevent mortality and morbidity. When the symptoms are manifested, it is often too late and result in severe mental and physical disability in what could have been a completely normal child. There is insufficient epidemiological data to prove the number of cases of IEM in India. The incidence also increases because of consanguinity which is prevalent in the country. Often the family suffers because, multiple progeny die from the same disease without proper diagnosis. Analysis of urine organic acid provides information on the metabolism. In this work a simultaneous GC-MS method for the determination of the levels of glutaric acid, methyl malonic acid, adipic acid, homogentisic acid and succinyl acetone in urine sample was developed and validated. These biomarkers were selected based on the fact that these are easily detected in urine and the conditions associated can be managed with suitable intervention if detected early. A simple extraction technique using ethyl acetate was employed and the derivatization was done in a single step with BSTFA mixture and the analyte in the ratio 1:1. The linearity, intra-day accuracy and precision were within the acceptable limits. The developed method was used successfully to quantify the organic acids in urine samples of paediatric patients.

P14. Selective Newborn Screening of Amino Acid, Fatty Acid and Organic Acid Disorders

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Mandatory newborn screening for metabolic disorders has not been implemented in most Middle Eastern countries. Preliminary studies conducted in some parts of Middle East suggest that the incidences of inborn errors of metabolism are reported to be higher in the Middle East than anywhere

else in the world due to the consanguinity. Results from a selective screening study to investigate the incidences of amino acid, fatty acid and organic acid disorders in dried blood spot samples from 1645 symptomatic children and based on the number of children born between 2008 to 2010 ($n = 51,925$) in Bahrain, suggest an alarmingly higher incidences of maple syrup urine disease ($n = 4$, 1:12,981), isovaleric acidemia ($n = 2$, 1:25,962), propionic acidemia ($n = 2$, 1:259,62), glutaric aciduria type II ($n = 3$, 1:17,308), methylmalonic acidemia ($n = 3$, 1:17,308) and arginosuccinic aciduria ($n = 1$, 1:51,925) compared to those reported in Europe and USA.

In this presentation results from this selective screening study are described and a need for an urgent mandatory mass-screening program in the Middle East and challenges ahead for implementation of newborn screening program in the region are addressed.

P15. An Improved Tandem Mass Spectrometry Method for GALC Enzyme Assay and Psychosine Analysis in Dried Blood Spots for Identification of Krabbe Disease

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Introduction: Krabbe disease is caused by deficiency of galactosylcerebrosidase (GALC) and the consequent accumulation of cytotoxic metabolite psychosine. Newborn screening was developed and conducted by using tandem mass (MS/MS) method. Now we developed a more accurate enzyme assay based on the enormous analytical range providing by MS/MS. We also measured the psychosine level in dried blood spot (DBS) for diagnosis and following up.

Method: GALC enzyme reactions were stimulated by optimal concentrations of additives in citrate-phosphate buffer, which was used to give maximum measurement of GALC activity. Psychosine was eluted with methanol containing internal standard. UPLC-MS/MS was conducted within two minutes for GALC assay and five minutes for psychosine analysis.

Result: Comparing to the sodium acetate buffer, citric phosphate buffer would activate the GALC enzyme activity 10 folds higher and inhibit the interference enzyme b-GAL activity. The P/IS response from Krabbe cells increased corresponding to the percentage of mixed wild type cells. The analytical range from 0.0003–0.7 nmol/h/mg protein was dose dependent and linear ($R^2 > 0.99$). Psychosine level was significant elevations in infantile patients. After the treatment, decreased psychosine was found in both infantile and late-onset patients, indicating improvement of disease severity.

Conclusions: The new MS/MS-based high accuracy GALC enzymatic assay provides a reliable method for diagnosis with clear distinction disease cells from wild type cells. Psychosine measurement could also serve as a second tier assay as a diagnostic tool and biomarkers for following up. Both of these rapid and highly sensitive methods are expected to reduce the number of false positives and to better predict the disease severity.

P16. Selective Newborn Screening for Inborn Errors of Metabolism in the Republic of Macedonia

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The development of electrospray tandem mass spectrometry (LC/MS/MS) in recent years has facilitated the introduction of expanded newborn screening programs in many countries, increasing the capacity to test newborns for rare metabolic disorders. LC/MS/MS can identify and quantify several acylcarnitines and amino acids in a single test, and is able to detect more than 40 inborn errors of metabolism, with the combined incidence of about 1 in 5000 babies, not including phenylketonuria (PKU).

Selective newborn metabolic screening in Macedonia was performed by measuring of two groups of compounds, 12 amino acids and 13 acylcarnitines, in dried blood spot (*n* = 4853) collected between 48 and 72 h of newborn life, using LC/MS/MS method, during the 2012–2015.

A total of 4853 newborns (5.27% of live born neonates in the study periods), selected from six birth centers in the country, have been screened. 2485 (51.2%) were male and 2368 were (48.8%) female with male to female ratio of 1.05:1. Among screened neonates 55.2% were Macedonians of Slavic origin, followed by ethnic Albanians (32.8%), Roma (6.4%), Turks (3.7%), Bosnians (0.7), Boshnjac (0.6%) and others (0.6%). Inborn errors of metabolism were detected in three newborns: phenylketonuria (PKU), hypermethioninemia (MET), and tyrosinemia type I (confirmed by the second tier test).

Newborn metabolic screening can provide substantial benefits to patients and their families if thoughtfully integrated into newborn screening programs, provided that sufficient funding is made available to cover the costs of additional and necessary personnel, medications, and medical foods. Activities to cover all newborns in Macedonia are underway.

Keywords: Metabolic disorders, Newborn screening, Tandem mass spectrometry

P17. Results of the Expanded Newborn Screening in Slovakia in Ethnic Majority and Roma Groups

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The authors present the first results of the national expanded newborn screening (NS) in Slovakia in majority (M) and roma (R) etnic population. Monitoring of the ethnicity was introduced since the striking ethnic differences in IRT screening test and low incidence of cystic fibrosis (CF) in roma newborns. Roma population represent app. 10% of total population, and roma natality is app. 15%. NS program was extended in 2013 of 20 hereditary metabolic disorders (HMD) using ms/ms.

Results: In 2013–2015 in total 165,648 newborns were investigated in NS (100% covering of population), 25,321 (15.3%) of them were the R ethnic group. 313 positive confirmed cases were discovered in the whole spectrum of detected disorders (total NS prevalence = 1:529, M = 1:758, R = 1:198, OR:3.83). Striking ethnic differences in NS prevalence were found in all spectrum of detected disorders. In roma ethnic group was slightly higher prevalence in congenital hypothyreosis (M = 1:2063 vs. R = 1:1332, OR: 1.35), only one case of CF, and no case of CAH was found in this ethnic group. In contrast to this, the total NS prevalence of HMD was expressively higher in R ethnic group (M = 1:1670 *vs.* R = 1:234, OR: 7.13). Striking were differences in the prevalence of individual types of HMD. Whereas the PKU and spectrum of aminoaciduria and organic acidurias dominate in M group, the fatty acids oxidation disorders (MCAD, SCAD) and carnitine defects (CUDD) were frequent in R newborn group.

Conclusion: Despite the presented results are preliminary, we can conclude that ethnic approach to NS allows us to record the striking differences in screening (and possibly real) prevalence of particular disorders, which would be missing during unitary approach. In addition this approach carries the new insights to Slovak population.

P18. Distribution, Incidence and Molecular Aspects of Target Diseases of Expanded Newborn Screening Using MS/MS in Japan

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Expanded newborn screening using MS/MS screening was initiated nation-wide from 2014 in Japan, after 16 years of pilot study from 1997 to 2012, in which a total of 1.95 million newborns were screened, and 217 cases (the overall incidence, 1 in 9000) with inborn metabolic disease (IMDs) were

detected. On the other hand, in Taiwan, which is located close to Japan, the overall incidence was around 1 in 7030 (1.39 million newborns tested during 2001 to 2014), whereas those in Germany was 1 in 2600 (6.11 million newborns during from 2004 to 2012).

The most commonly detected disease in Japan was propionic acidemia (PA), at 1 in 45 thousands (45 K), followed by PKU (1 in 53 K), citrin deficiency (CTL-2) (1 in 85 K), MCAD deficiency and methylmalonic acidemia (1 in 110K, each). In Taiwan, that was MCC deficiency (1 in 49 K), followed by PKU (1 in 58 K), and CTL-2 (1 in 61 K), while in Germany, that was PKU (1 in 5 K), followed by MCAD deficiency (1 in 10 K), and CTL-1 (1 in 60 K).

In molecular aspects, half of Japanese PA cases are mild form that has a Japanese specific common mutation on PCCB (1304T>C). In Japanese MCAD deficiency, c.449-452delCTGA, which covers about 45% of alleles, was found, although 985A>G, which is a common mutation of Caucasians patients, was never found. In 50 Japanese cases with VLCAD deficiency, 12% (6/50) was severest form; 8% (4/50) was intermediate form. In contrast, 46% (25/54) was severest cases, and 39% (21/54), intermediate form in Caucasian population. In Japanese TFP deficiency, 80% (12/25) were HADB deficiency, whereas in Caucasian population, 90% (46/52) were HADA deficiency in which c.1528G>C was identified in 53/104 alleles (52%). The disease distribution, incidence, and molecular aspects appear to be different country to country.

P19. MCAD Deficiency with Severe Neonatal Onset, Fatal Outcome, and Normal Acylcarnitine Profil

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Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is an autosomal recessively inherited disorder of fatty acid oxidation with potentially fatal outcome in undiagnosed patients. Introduction of tandem mass spectrometry into newborn screening (NBS) has led to the inclusion of MCADD into NBS in many countries, which has resulted in a significant reduction of morbidity and mortality. We report a child with MCADD presenting neonatally with apnoea and heart arrest. Despite intensive efforts to rescue the child, including reanimation for 90 min, the child died at the 2nd day of life. Autopsy revealed fatty liver and also fat storage in heart muscle, which was suggestive for a fatty acid oxidation defect. However, acylcarnitines determined from stored EDTA blood were not suggestive for MCAD deficiency. Nevertheless, a subsequent whole exome sequencing analysis revealed homozygosity for the ACADM gene c.1084A>G/p.Lys362Glu mutation.

P20. Age-Related Variations Study of Blood Amino Acids Concentrations in Premature Neonates. High Incidence of Elevated Tyrosine

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Age-related variations of amino acids concentrations are important for the clinical assessment of inborn errors of metabolism in newborn screening. Previous investigations reported in literature were focused on plasma amino acids. We have performed a small study on blood samples collected from premature neonates at different sampling dates, in order to improve the evaluation of screening results in specific cases (non-standard newborn screening).

The study was conducted at Alfred Rusescu; Institute for Mother and Child Care, Polizu Maternity, Neonatology Clinic, with the approval of the Ethic Committee. Thirty premature newborns (gestational age 23–33 weeks) were included in the research. Blood samples were collected from the umbilical cord, and from the neonate after 24 h, 72 h, 7 days and 4 weeks after birth. Dried blood spots were processed with the standard method for amino acids and acyl carnitines analysis (Chromsystems MassChrom

kit). Concentrations were measured on an LC-MS/MS system composed by an Agilent binary pump and autosampler, connected to a Sciex 3200 triple quadrupole mass-spectrometer.

Elevated tyrosine concentrations, 1.2 up to 5 fold higher than the reference range, were measured at 72 h and/or 7 days in 14 subjects (46.6%). All values normalized 4 weeks after birth. This indicates a high rate of transient tyrosinemia in pre-term babies. These elevations are not correlated with the gestational age or type of pregnancy (single or multiple with twins or triplets). Age-related variations were also observed for arginine and citrulline (urea cycle disorders markers), while phenylalanine, leucine+isoleucine, glycine, glutamic and aspartic acids were not influenced significantly. These results are helpful in the routine clinical assessment of inborn errors of metabolism.

P21. NeoBase™2 Non-Derivatized MSMS Kit—Improved Performance for Expanded Newborn Screening

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The PerkinElmer® NeoBaseTM (NB1) newborn screening kit uses a non-derivatized MSMS method to measure blood levels of amino acids, free carnitines and acylcarnitines in newborns. Introduced in 2006, abnormal levels determined by the kit may be indicative of inborn errors of metabolism. The next generation of NeoBase kit, NeoBase™2 (NB2), is being developed to improve analyte detection, laboratory productivity and instrument compatibility. In this development, assay functionality is increased by including additional small molecule markers of inborn errors of metabolism to the current NB1 panel. The NB2 kit is proposed to measure all NB1 analytes plus four new amino acids—arginine (Arg), ornithine (Orn), glutamine (Gln) and argininosuccinic acid (ASA); five new acyl carnitines—C18:2OH, C20, C22, C24 and C26; four new lysophospholipids C26:0 LPC, C24:0 LPC, C22:0 LPC and C20:0 LPC; and two purine nucleosides adenosine (ADo) and deoxyadenosine (dADo). The literature suggests that abnormal levels of one or more of these markers may be associated with OTCD (ornithine transcarbamylase deficiency) diseases, ASALD (argininosuccinic acid lyase deficiency), X-ALD (X-linked adrenoleukodystrophy) and ADAD (ADA-SCID, Adenosine Deaminase Deficiency). Compared to some other methods the NB1 kit has somewhat lower recovery of succinyl acetone and longer sample preparation time. Based on preliminary development work, succinyl acetone's recovery can be enhanced while sample preparation time can be reduced significantly to 90 min from 165 min. The concentration of the marker analytes spiked into the low and high control dried blood spots (DBS) are being updated to provide levels more representative of the concentrations with clinical relevance. The development goals of the NB2 project are based upon nine years of usability from the NB1 globally sold product. The NB2 project aims to incorporate new markers, improved performance and enhanced functionality.

P22. Performance of an Fia-MS/MS Method to Simultaneously Measure ABG, ASM, GAA, GALC, GLA, and Idua Activities from a Single 3.2 mm DBS Punch

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A new multiplex flow injection analysis—tandem mass spectrometry (FIAMS/MS) method is described that simultaneously measures the activities of the enzymes ABG, ASM, GAA, GALC, GLA and IDUA, using a single 3.2 mm punch from a dried blood spot (DBS). Other MS and fluorescence methods require six separate incubation cocktails to measure the same activities. Compared to currently available MS reagents, novel structural modifications include: ABG and GALC substrates (S) altered to improve solubility, IDUA-S made similar to GAA-S and GLA-S, and internal standards (IS) for

ABG, ASM, GALC and IDUA are deuterium-labeled versions of the enzymatic products. Furthermore, the reagent buffer is optimized for a single cocktail six-plex format.

P23. Benefits of Determination of Total Homocysteine, Methylmalonic Acid, and 2-methylcitric Acid in Dried Blood Spots for Newborn Screening and Confirmatory Testing: The Qatar Experience

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Background: Abnormal findings for propionylcarnitine (C3) and methionine using MassChrom® Amino Acids and Acylcarnitines from dried blood spots (DBS) can be attributed to a number of different inborn errors such as homocystinuria, remethylation, and cobalamin deficiency. Although the incidence of homocystinuria in the Qatari population one of the highest among the world, 1:1,800, and about 6% of the population are carriers, we cannot exclude the other inborn errors. Both methionine and C3 are not specific for these conditions which lead to increased false positive results, causing parental anxiety, need for further metabolic investigations, and greater follow-up costs. Useful additional markers such as total homocysteine (tHCY), methylcitric acid (MCA), and methylmalonic acid (MMA), are not detected by MassChrom® Amino Acids and Acylcarnitines from DBS. To improve diagnostic specificity, we validated and implemented a 2nd tier method using liquid chromatography–tandem mass spectrometry (LC-MS/MS), for the detection of tHCY, MMA, and MCA in DBS.

Method: Full validation and implementation since November 2015 of 2nd tier test to measure quantitatively tHCY, MMA, and MCA in DBS. Altogether the method yields diagnostic values for methionine, C3, tHCY, MMA, and MCA.

Results: Review of the diagnostic values for the 2nd tier analytes along with the traditional screen results in a significant decrease in the number of false positive screens as well as more rapid and targeted recommendation for confirmatory tests for presumed positive screens.

P24. Newborn Screening for Arginase Deficiency in the U.S.—Where Are We?

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Arginase controls the final step of the urea cycle, which produces urea by removing nitrogen from arginine. When arginase is damaged or missing, arginine is not properly broken down. The net result of arginase deficiency is failure to produce urea normally, and the accumulation of arginine and its metabolites. Failure of early detection and delayed institution of clinical management frequently results in life long mental and physical disability. When arginase deficiency is detected early, patients can be more effectively managed and the effects of the disease mitigated. Enzyme replacement therapies are in development.

Currently arginase deficiency is recommended as a secondary target for newborn screening in the U.S. Elevation to a "core" condition would result in more babies being screened, diagnosed and treated. In order to nominate the condition for formal evidence review and consideration, it is useful to know the current status and issues relative to screening implementation in U.S. programs. In this regard, we have reviewed the arginase deficiency screening activities of 51 U.S. programs, including all states and the District of Columbia. While many programs either require or include detection of the condition as a result of their screening protocol, some do not. Additionally, the laboratory methodologies vary widely among those that include it and are often suboptimal.

We review the U.S. national data on current screening activities for arginase deficiency with particular attention to laboratory screening protocols, including analytes of interest, cutoffs, ratios, and comment on possible protocol improvements to advance early detection and minimize disease morbidity and mortality.

P25. Determination of Butyrylcarnitine/Isobutyrylcarnitine (C4) by Tandem Mass Spectrometry: Diagnosis of SCADD in Newborn Screening in Slovakia, Geographical and Ethnic Specialties

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On this sheet we presented an application and results obtained by tandem mass spectrometry MS/MS for determination of butyrylcarnitine/isobutyrylcarnitine (C4), which accumulation can predict a metabolic disorder called Short-chain acetyl-CoA deficiency (SCADD). Determination of C4 is performed from dry blood spot (DBS) using a kit from Chromsystems MassChrom. Amino Acids and Acylcarnitines from DBS by LC-MS/MS, and identified by isotope dilution mass spectrometry methods (MRM transmition of C4 288.2 -> 85.0) using Agilent 6420 Triple Quadrupole LC/MS. Within 2015 in Slovakia there were 55 694 newborns, all of them were screened for metabolic disorders (including SCADD). Out of 153 first positive samples for C4 there were 78 cases of repeatedly increased C4 above the cut off (0.95 µmol/L). In the context of positive cases there were 65 newborns belonging to the Roma ethnic group of which has 5 positive genetic mutation for SCADD for the moment, 13 with Caucasian ethnicity, 23 newborns with birth weight under 2500 g, 14 newborns under gestational week 36 and 7 cases of exitus (all of them was Caucasian ethnicity). Within the framework geographical distribution of Slovakia there were found 68 cases of positive C4 located on the East part of Slovakia. Tthis distribution of SCADD closely reflects the distribution of Roma population in Slovakia. Based on these results obtained in year 2015, we would like to include SCADD for NBS in Slovakia.

P26. Outcomes of Patients with Cobalamin C Disease Identified through Newborn Screening: A 16-Year Experience

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Cobalamin C (CblC) disease is the most common inborn error of cobalamin metabolism. Newborn screening (NBS) for this disorder was implemented in our referral area 16 years ago. Over this time period, 12 patients have been identified through screening, they now range in age from 4 months to 16 years. The average age at diagnosis was 8.1 days, and therapy was initiated immediately after diagnosis in each infant. 11/12 (91.7%) of the infants were symptomatic and hospitalized at the time of diagnosis. Symptoms included poor feeding, acidosis, and hypoglycemia. Mean initial C3 level was 9.17 µmol/L (normal < 1.6), mean initial total homocysteine was 151.9 µmol/L (normal < 15), and mean initial methionine level was 7.53 nmol/L (normal > 8). All infants were started on therapy with hydroxycobalamin, betaine, and a low protein diet with methionine supplementation as required to maintain normal serum levels. However, despite compliance with therapy and improvement in biochemical markers most patients still experienced significant morbidity. The children were admitted to the hospital an average of 2.2 times each after the neonatal period. 3/15 (25%) of patients were microcephalic at last evaluation. 8 individuals underwent brain MRI and 4 (50%) demonstrated abnormalities including prominence of the ventricular spaces, volume loss and white matter changes. Formal neurocognitive testing was completed in eight children, and 7/8 (87.5%) showed moderate or severe cognitive impairment. One individual with mild biochemical abnormalities at presentation had a normal neurocognitive evaluation. 8/12 (66.7%) subjects developed nystagmus, while retinal abnormalities were detected in 7/10 (70%) of those examined. This population demonstrates that despite early identification and initiation of therapy, individuals with CblC are at high-risk of neurocognitive and ocular complications of disease.

P27. An Analysis of Newborn Screening for Galactosemia and Genotype—Phenotype of Confirmed Galatosemia Cases

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Objective: To investigate the prevalence of galactosemia (GAL), and the characteristics of genotype and phenotype of newborns who were confirmed with GAL in newborn screening in Zhejiang province. Method The number of all live births, newborn screened infants and all clinical data of confirmed newborns with GAL from October 2013 to March 2015 were retrospectively analyzed by reviewing the data of Zhejiang Province screening center database. And the characteristics of genes and the clinical data of GAL cases who were confirmed by correlative gene test and enzyme activity measurement were analyzed.

Results: Among 759,428 babies did newborn screening of GAL, 4 cases confirmed, the prevalence of GAL in Zhejiang province is 1/189,857. Among them, 1 case was confirmed with GAL type I, with mutations of c.904+1G>T(splicing)and c.687G>A (p.K229K),the enzyme activity of galactose-1-phosphate uridyltransferase (GALT) was 56.4% of controls. And there was 1 case of GAL type II, with mutations of c.85G>T (p.E29*) and c.502G>A (p.E174K). Her elder sister was confirmed as the same genotype patients, who had cataract surgery twice due to congenital cataract, without GAL newborn screening. There were 2 cases confirmed with GAL type III, with mutations of c.505C>T (p.R169W), c.452G>A (p.G151D), c.280G>A (p.V94M) and c.925G>A (p.A309T), the enzyme activity of UDP-galactose-4"-epimerase (GALE) were 42% and 38% of controls, respectively. All cases had different abnormal biochemical marks of liver function, and 1 case combined with hyperlactacidemia or hyperammonemia or increasing of multiple kinds of amino acids, respectively. The newborn of GAL type II had phacoscotasmus before treatment.

Conclusion: The disease of GAL is rare in Zhejiang province, and its gene distribution is scattered with comparatively mind clinical manifestations, and the cases who are confirmed in newborn screening with early treatment by lactose free milk powder have good prognosis.

P28. Very High Incidence of Low Vitamin B12 in Estonian Newborns

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The worldwide spread of newborn screening programs has greatly contributed to highlight the incidence of cobalamin deficiency, even in industrialized countries. It is suggesting that vitamin B12 deficiency is more common than previously thought.

A population-based extended newborn screening by using tandem mass spectrometry was introduced in Estonia 1 January 2014. Over the past two years we have screened 27,369 children and diagnosed 11 newborns with vitamin B12 deficiency. All of them had elevated C3 levels (>4.31 µmol/L) and a C3 to acetylcarnitine ratio (C3/C2) of 0.2 or greater. During that period we have adjusted the cut-off level of C3 to improve the identification of vitamin B12 deficiency in newborns.

Every identified child underwent paediatric evaluation and the further laboratory testing, including the measurement of vitamin B12, folate and homocysteine in serum, and methylmalonic acid content in urine. All 11 newborns were asymptomatic when treatment was initiated, and subsequently all biochemical markers normalized after treatment.

Most of the mothers, except two, had normal levels of vitamin B12. One mother with low cobalamin was mainly vegetarian and another had autoimmune disease, which may explain vitamin B12 deficiency in their children. All the other mothers were healthy and the cause of their children's low cobalamin remained unclear.

In conclusion: the incidence of congenital acquired vitamin B12 deficiency is high (40.2/100,000 live births). Our study supports that vitamin B12 deficiency occurs more frequently than is currently recognized. Based on our experience, the cause of it persists hidden.

P29. Performance of a Four Step Screening Strategy for Cystic Fibrosis during the First Years in the Dutch Routine Newborn Screening Program

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Background: Since 1 May 2011, newborn screening for Cystic Fibrosis (NBSCF) is part of the Dutch routine newborn screening program, using a 4-step strategy (Immunotrypsinogen (IRT)/Pancreatitis-associated Protein (PAP)/Line probe assay (InnoLiPA) for 35 CFTR mutations/extended gene analysis (EGA) (Thorax 2012; 67, 289–295) with the primary aim to identify patients with classical CF. Newborns with one or two mutations leading to severe CF, or two mutations of unknown clinical relevance are referred to a designated CF centre for further diagnosis. Healthy carriers and newborns with an inconclusive diagnosis (CFSPID) are considered false positives.

Aim of study: to assess the performance of the screening strategy when applied in a routine screening program.

Methods: Laboratory data were collected from 1 May 2011 to 1 January 2015. CF centres reported results of sweat tests in the NBSCF register, and CF patients not identified by NBSCF to the Dutch Paediatric Surveillance Unit.

Methods: 647863 newborns, 99.4% of all newborns, were screened for CF; in 6484 samples IRT was > 60 µg/L, after PAP analysis InnoLiPA followed in 807; 97 samples had 2 CFTR mutations; EGA analysis was performed in 64 samples with 1 CFTR mutation, and in 363 samples as safety net. 159 newborns were screen-positive. CF was confirmed in 97, we found 27 CFSPID and 35 healthy carriers. A false negative screening test was reported for 13 (4 with meconium ileus (MI)) CF patients. Sensitivity of NBSCF was 91.5% (excluding patients with MI), specificity 99.99% and positive predictive value (PPV) 61%.

Conclusion: Compared to other NBSCF programs, the high specificity, PPV and low number or referred carriers of the Dutch program are excellent but the sensitivity does not meet standards of care (JCF 2014;13, S23-42). Alternative cut-off values for PAP and another approach of the safety net may increase sensitivity.

P30. CF Screening in Manchester, UK: A Review of Cases Detected from a Repeat IRT Taken at Day 21–28

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Background: There is no universally-agreed approach to screening for cystic fibrosis (CF). In the UK the first step is the IRT assay followed by one or two-stage mutation analysis of the CFTR gene on all samples with IRT values > 99.5th centile (cut-off 1). If one or no mutations are detected and the IRT is > 99th centile a second IRT test is undertaken on a repeat dried blood spot sample (taken on day 21–28). If the second sample IRT is > cut-off 2 (10 ng/mL below cut-off 1) the baby is referred to the CF clinical team with a presumptive positive diagnosis for evaluation (clinical assessment, sweat test, further mutation analysis). The purpose of this procedure is to detect cases with mutations not covered by the panels currently in use.

Objectives: To review the number of babies diagnosed with Cystic Fibrosis following referral from the Manchester Newborn Screening Laboratory as a result of second IRT testing from November 2007–April 2015.

Methods: Presumptive positive babies with one or no mutations detected were identified from the Newborn Screening information System (Specimen Gate, Perkin Elmer). Clinical data on these babies was provided by the CF clinical team.

Results: 421,358 babies were screened from November 2007–2015 and 160 in total were reported as CF suspected (I in 2633). Of these 35 were referred following second IRT testing (10 with 1 mutation and 25 with no mutations). A diagnosis of CF was confirmed in 12 babies (6 with 1 mutation and 6 with no mutations); all had sweat chloride levels > 60 mmol/L and additional genetic mutations detected as a result of further mutation analysis/gene sequencing. In 2 further screen positive babies with one mutation the diagnosis was made prior to screening. Two babies with atypical CF and normal sweat chloride levels were also detected.

Conclusion: An additional 12 babies with confirmed CF were detected following second IRT testing and will benefit from early intervention and follow-up. This provides supporting evidence for the continuation of this component of the screening protocol.

P31. A 7-Year Review of cf Newborn Screening Results from a UK Regional Laboratory

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Introduction: Newborn screening (NBS) for cystic fibrosis (CF) was implemented across the whole of the United Kingdom by July 2007. The West Midlands Newborn Screening Laboratory is one of 16 screening laboratories in the UK and serves two tertiary paediatric CF centres. We report the CF NBS results from this laboratory.

Methods: The CF NBS results from the West Midlands Newborn Screening Laboratory were reviewed from 1 November 2007 to 31 October 2014.

Results: In total 507,608 babies were screened. 200 were referred to one of the tertiary CF centres as CF Suspected; 165 were subsequently confirmed as CF, giving birth prevalence of 1 in 3076 live births and a positive predictive value of 82.5%. Of these, 83/165 (50.3%) were homozygous and 63/165 (38.1%) were heterozygous for Phe508del. 101 carriers were identified. To date, 9 patients who had a negative CF NBS within this time period have been diagnosed with CF, one of which had meconium ileus.

Conclusions: The birth prevalence of CF in the West Midlands is lower than the expected figure for the UK which is 1 in 2381. This difference is likely to be due to two factors; the ethnic diversity of the West Midlands population and children with negative screening who are yet to be diagnosed. In keeping with the aims of the UK CF NBS program the rates of carrier detection and false negatives were low. We now plan to review the case notes of all patients with a positive CF NBS to gain anthropological and microbiological data for the first 2 years of life.

P32. Final Results of the Pilot Study for Cystic Fibrosis Newborn Screening in Portugal

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Cystic Fibrosis (CF) is the most common life-threatening monogenic condition in Caucasians, affecting more than 30,000 individuals in Europe. It is a complex disease with multi-organ involvement and large clinical variability, although the most frequent cause of morbidity and mortality is lung disease. The early implementation of therapy to asymptomatic cases identified through newborn screening (NBS), proved to result in a undoubtedly benefit to the newborn.

A pilot study for CF newborn screening (NBS) was conducted in Portugal, between October 2013 and December 2015. This study included 183,000 newborns, born in the whole country within this time. An IRT/PAP/IRT strategy was used, with 1st IRT (Normal < 65 ng/mL) and PAP (Normal < 1.6 ng/mL if 65 ng/mL $\leq$ IRT < 100 ng/mL or Normal < 0.5 ng/mL if IRT > 100 ng/mL), measured in the newborn screening sample, usually taken between the 3rd and 6th days of life. Among all the screened newborns, 1577 (0.9%) presented an elevated first IRT value, but only in 615 (0.3%) cases a 2nd sample was requested, due to IRT $\geqslant$150 ng/mL or combined elevation of IRT and PAP values. Sixty-two newborns maintained an elevated IRT (Normal < 50 ng/mL) in the 2nd sample, taken between 3rd and 4th week of life, and were sent to a specialized clinical center for sweat test, clinical evaluation and genetic analysis. Twentysix of these cases were confirmed to be CF patients, indicating a prevalence for this condition of 1:7038 newborns. Mutation p.F508del was found in 83% of the patients' alleles, revealing a high frequency in Portugal. CF screening should be soon integrated in the Portuguese NBS panel and the strategy tested in the pilot study should be maintained.

P33. Newborn Screening for Cystic Fibrosis in Switzerland—Evaluation after 5 Years

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Newborn screening (NBS) for cystic fibrosis (CF) started in January 2011 in Switzerland, after application to the Health Ministry and thorough evaluation of the screening protocol. One part of the approval is the obligation of a yearly evaluation. Regular evaluations have resulted in improvement of the CF-NBS in Switzerland. The Swiss CF screening uses an IRT/DNA protocol including 18 of the most prevalent mutations in the CFTR gene, and a safety net, in order not to miss classical CF patients of non-Swiss origin. Out of 420,731births within 5 years, 443 children were screened positive and referred to a CF-centre. 120 (27%) were diagnosed with CF, 18 (4%) had CFSPID, 302 (68%) children were CF negative, and 3 (0.7%) lost to follow up. Furthermore, 7 children with negative screening result were later diagnosed with CF (5.5% false negatives = 7/127). The sensitivity of the test was 94.5% (120/127). The PPV of CF-NBS testing was 27.1% (120/443); or 31.2% (138/443) when CFSPID cases were included. The specificity (420,281/420,604) and NPV (420,281/420,288) reached almost 100%. After 5 years, the NBS for CF remains successful in detecting nearly all children with CF. In order to improve the PPV of the screening test, pancreatitis-associated protein (PAP) measurement as a further technique is under evaluation.

P34. The Prevalence of Hereditary Hemoglobin Disorders and Its Implications for Newborn Screening in Germany

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Background: Some hemoglobin disorders are among those hereditary diseases with evidence that an early diagnose and treatment improves the clinical outcome of affected children. So far hemoglobin disorders are not included in the German newborn screening program despite increased immigration from countries with populations at risk.

Design: To determine the birth prevalence in a major German metropolitan area we tested 17,018 newborns. High pressure liquid chromatography (HPLC) and subsequent molecular-genetic testing were used for the detection and confirmation of hemoglobin variants.

Results: Out of relevant diseases the most prevalent was sickle cell disease with a frequency of disease-consistent genotypes of 1 in 2385 newborns. Duffy-bloodgroup typing showed evidence that 90% of the affected children were likely of sub-Saharan ancestry.

Conclusion: Sickle cell disease (SCD) was found as prevalent as hypothyroidism, the most common disorder of the German routine newborn screening program. An inclusion of SCD into NBS seems reasonable from medical and ethical viewpoints.

P35. Development of a Diagnostic Kit for Targeted Screening of Hemoglobinopathies

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Objective: We applied a methodology based on the use of Tandem Mass Spectrometry (MS/MS) to detect all kinds of hemoglobinopathies (qualitative abnormalities and α- and β-thalassemias) in only one run, during less than 2 min. To fit the requirements for NBS programs, the analysis is based on the use of DBS samples.

Material & Methods: The sample preparation was optimized for DBS punches of 3.2 mm and includes only 4 steps: extraction of Hb with water, denaturation of Hb with ACN/FA 1% 50:50, digestion with trypsin (37 C, 2 h, 650 rpm) and dilution in solvent compatible with MS. The extract is injected in a triple quadripole instrument in a flow injection analysis mode (using a UPLC sample manager). The instrument works in Multiple Reaction Monitoring (MRM) mode (3 transitions per peptide) and the areas of peak of peptides of interest are measured. The calculation of ratios of intensity between specific peptides, allied to a decision tree, allows to diagnose each type of hemoglobinopathy.

Results: We performed a preliminary validation of our methodology on samples coming from the Laboratory of Genetic Biochemistry of Liège University Hospital Center. 250 samples were healthy, 6 were coming from patients carrying a mutation of β-chain and 4 were coming from premature babies.The analysis of the 250 samples allowed us to calculate the cut-off values for peptide intensity ratios. Using these cut-off values, we were able to determine correctly the type of mutation for each of the 6 unhealthy samples, which were 2 HbAS, 2HbSS, 1 HbAC and 1 HbAE. As expected, the results for premature babies' samples were all below the cut-off value for both quantitative and qualitative abnormalities, reflecting the low quantity of β-chain in premature babies' blood.

Conclusion & Perspectives: A clinical validation will be performed on a larger scale, including samples from patients suffering of α-thalassemia. After that, the developed methodology will be applied in an IVD kit providing all the constituents required for the analysis of 960 DSB samples.

P36. Evaluation of the Clinical Utility and Cost Effectiveness Ratio of Generalized Neonatal Screening for Severe Combined Immunodeficiencies (SCID) by Quantification of Trecs on Guthrie Cards: Preliminary Results

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SCID is asymptomatic at birth, but fatal within the first year of life. The confirmation of the disease is easy (numeration of lymphocytes subsets), there is a curative treatment, and it is known that early treatment improves survival. Therefore SCID follows criteria for population-based newborn screening (NBS). Quantification of TRECs has been proposed for that. We study the feasibility and cost-effectiveness ratio of generalized neonatal screening for SCID by offering it to 200,000 children over France. Prospective control group consists of children diagnosed with SCID out of 700,000 annual births who do not benefit from screening.

The protocol is leant against the existing NBS. TREC quantification is performed using the commercial Enlite kit from PerkinElmer. It is a multiplex analysis with amplification of TRECs and Beta-actin as the reference gene. Cut-off values were proposed after having tested 3000 anonymous newborns. If the result is below the cut-off value (20 TRECs copies/μL), the screening is declared positive or inconclusive (if the reference gene is not amplified), if it is above the cut-off value, it is declared negative.

For babies born at term, if the screening is positive, the pediatrician is informed and calls the parents for a visit and a flow cytometry analysis. If the result is inconclusive or in pre-term babies, a new dried blood sample (DBS) is requested.

Results on the first 52,374 samples show a recall rate of 0.31% (0.19% for a new DBS, 0.12% for a visit). The median of TREC copies is 116 copies/μL. 7 babies were included in the control group at that stage. The maximum TREC value observed was 11.

In conclusion, we observe:

1. A high recall rate for a visit
2. SCID patients have no or low TRECs
3. No SCID in the babies recalled for a visit.

We suggest a new algorithm with an equivocal zone between 11 and 20 in which we will request a new DBS instead of requiring a visit. Then if the screening on the second DBS is still positive, we will request for a visit. This algorithm will be confirmed according to currently on-going analysis on the first 100,000 samples.

P37. Homogeneous Single-Point PCR Detection of TREC and Beta-Actin DNA in Dried Blood Spot Samples

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Simple methods are needed to perform genetic tests. Many of the homogeneous PCR assays require either DNA extraction from dried blood spot (DBS) samples or other pre-treatment steps of the DBS samples to perform the analysis. Our aim was to study whether multiplexed detection of T-cell receptor excision circles (TREC) and beta-actin DNA is feasible from DBS samples with minimal sample pre-treatment.

A 1.5 mm disk sample was punched into the reaction well and elution incubation was performed. This was followed by the addition of the PCR mix. The reactions were sealed and moved to a thermal cycler. After cycling, the amount of TREC and beta-actin amplicons was measured through the seal without removing the disk. The methodology utilizes dual-label time-resolved fluorescence resonance energy transfer as detection chemistry. Each experiment contained negative (no template) and positive control reactions.

The influence of storage time, temperature, and humidity on TREC concentration was studied using DBS samples. Storage of specimens in elevated temperatures and humidity increases the risk of false positive TREC screening results. Additional attention should be given in these storage conditions.

Our findings suggest that DBS is a suitable matrix for an extremely simple method to perform multiplexed single-point PCR measurement. The analysis of known and unknown DBS reference materials showed that TREC and beta-actin amplification products may be successfully and correctly detected. To respond to the requirements of screening laboratories, we present that DBS may be used as a sample matrix in PCR with very simple pre-processing of the sample disks.

P38. Newborn Screening and Diagnosis of Lysosomal Storage Diseases

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We have developed mass spectrometry for newborn screening of several lysosomal storage diseases. In the WA state NBS lab we have completed a pilot study of the new University of Washington/Perkin Elmer 6-plex flow injection-tandem mass spectrometry assay for Gaucher, Pompe, Fabry, Niemann-Pick-A/B, MPS-I and Krabbe diseases. The assay is easy to execute and gives a significantly lower false positive rate compared to other methods (fluorescence).

More recently we have developed a multiplex assay using tandem mass spectrometry for additional lysosomal storage diseases: MPS-II, IIIB, IVA, VI, VII, and NCL2. These are done with a single dried blood spot punch, a single incubation cocktail, and a single infusion into the mass spectrometer. This assay is being piloted in the WA state NBS lab.

We have developed an assay of sulfatides in dried blood spots for newborn screening of metachromatic leukodystrophy. A pilot study in the WA state NBS lab is starting in early 2016. This is in response to recent treatment success for MLD using stem cell transplantation.

We have pursued UHPLC-tandem mass spectrometry for ultra-accurate assay of lysosomal enzymes in white blood cells to help improve the prediction of lysosomal storage disease severity. This is an important development since the results of newborn screening programs are often not definitive based on the primary screen even when combined with genotype determination. This has been particularly an issue with Pompe and Krabbe diseases. We can readily distinguish early versus late onset Pompe disease by UHPLC-MSMS. Results for Krabbe disease look promising so far, but more data is needed for a definitive conclusion.

P39. Multiplex Screening for Treatable Lysosomal Storage Diseases (LSDs)

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Introduction: The interest in neonatal screening for LSDs has increased substantially because the need for early diagnosis improves the therapeutic efficacy of new developed treatments and because screening has been made possible by recent technical advances. The results of pilot LSD screening studies (USA, Austria, Taiwan) show that the current clinical prevalence is underestimated.

Method: Tandem mass spectrometry (MS/MS) has become an established tool for the detection of rare congenital metabolic disorders by newborn screening. The LC-based method has advantages for expanding the assays to include additional products and internal standards for multiplexing all nine currently available lysosomal enzyme assays in dried blood spots (Gaucher, Niemann-Pick A/B, Fabry, Krabbe, Pompe, MPS-I, MPS-II, MPS-IVA and MPS-VI) as well as allowing other metabolites to be quantified (Spacil et al. 2012, Clin Chem).Validation of this method to the full set of treatable lysosomal storage disorders has been performed in our laboratory including improvement of the enzyme assay for Gaucher.

Results: ABG, GAA, GLA and IDUA enzyme activities in over 27,000 newborn samples were analyzed. Statistically significant higher GAA and GLA enzyme activities were observed in female newborns compared to male newborns. And newborns with a higher birth weight and gestation age have a statistically significant lower GAA and GLA enzyme activity compared to newborns with lower birth weight and gestation age. This proofs that it is of importance to define the reference intervals for lysosomal enzyme activities as well as cut-off limits for newborn babies with regard to birth weight, gestational age and sex for each population. True positives were not yet found.

Conclusion: We report for the first time results of a 6-plex enzymatic activity UHPLC-MS/MS assay for the lysosomal storage disorders Gaucher, Fabry, Pompe, MPS-I, MPS-II, MPS-IVA and MPS-VI in Europe. This method is accurate, fast, has low cost and is easy to implement next to the routine newborn screening and therefore suitable for LSD newborn screening.

P40. Newborn Screening for Mucopolysaccharidosis Type II in Taiwan

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Mucopolysaccharidosis type II (MPS II) is an X-linked recessive lysosomal storage disease caused by deficiency of iduronate-2-sulfatase (IDS). As better outcomes are related to early treatment, prompt diagnosis is critical with the availability of treatment, indicating a need for newborn screening (NBS).

We report the results of a large scale pilot NBS program, measuring IDS activity by LC-MS/MS in dried blood spots (DBS). In the pilot study, DBS quality controls from the US CDC were used to validate the precision of the method. Eleven confirmed positive patients and 3052 anonymous DBS were collected for generating reference value. Afterwards, a total of 28,814 newborns participated in the program from August to December 2015. Mutation analysis was further performed for those screened twice with enzyme deficiency.

The method showed acceptable precisions with intra- and inter-assay coefficients of variation being 14.0% to 16.1% for the controls. The cutoff value was set at 30% of the average enzyme activity (6.5 μmol/h/L), giving a recall rate of 0.16%. All positive cases fell in the suspicious range. Of the 28814 tested, 53 (51 males, 2 females) underwent mutation analysis. We found 3 undescribed and 2 reported non disease-causing mutations; they were c.103+34_56dup, p.T500I (reported), p.R101C (reported), p.R297H, and p.Q200L. Notably, the previous three accounted for 91% of all alleles, with each shared one third; moreover, they expressed 5%, 10% and 19% of the average activity, respectively. All the suspicious individuals were referred to hospital for following up.

It is feasible to use LC-MS/MS measuring IDS activity for NBS. Adjusting cutoff value to lower recall rate is recommended. The role of the mutation c.103+34_56dup in MPS II pathogenicity needs further study.

P41. Feasibility Study Using the Six-Plex Perkinelmer LSD Reagents Utilising Tandem Mass Spectrometry (MSMS) for Newborn Screening and Diagnostic Testing for Six Lysosomal Storage Diseases (LSD)

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Lysosomal storage disorders (LSD) are a group of more than 40 disorders caused by the specific deficiency of enzymes (& co-factors) within the lysosome. The estimated incidence in the Australian population is 1 in 7000 (Meikle et al. JAMA 1999).

The 6 LSD enzymes were galactocerebrosidase (GALC; Krabbe disease), acid a-galactosidase A (GLA; Fabry disease), acid sphingomylinase (ASM; Niemann Pick A/B disease), a-iduronidase (IDUA; mucopolysaccharidosis type I) and b-glucocerbrosidase (ABG; Gaucher disease). De-identified newborn dried blood-spots (DBS) were sourced from the South Australian population & from confirmed positive LSD cases (N = 75) that included GALC (5), GLA (14) & carriers (28), ASM A/B (1), IDUA (6), GAA (20) and ABG (20). Individual LSD enzyme activities were determined by stable isotope dilution technique by MRM after a single organic solvent extraction and determination by FIA on an API5000 MSMS. MSMS setting were, IS voltage 5000, DP 95, CE 55 & CXP 15. All six LSD assay performance had CV% on repeat DBS QC analysis of < 12%. The normal newborn population gave the 1st centile in IU/h/L whole blood for each enzyme as ABG 2.2, IDUA 1.3, GAA 4.1, ASM 0.8, GALC 1.8 & GLA 1.3. These were used to assess known positive LSD DBS. A comparison between LSD activities measured in leucocytes using the standard 4-MU fluorogenic method and dried blood-spots using the 6-Plex PE MSMS showed excellent correlation. All LSD cases had activities below the < 1st centile of the respective normal population, GLA showed some overlap, due to the non-enzymatic in-source fragmentation of the substrate, this was specific to the API5000 instrument. Each assay showed > 3 orders of magnitude in dynamic range with excellent lower end sensitivity.

Our study clearly showed the superior performance with large dynamic range and lower end assay sensitivity that was able to distinguish between unaffected and confirmed true positive LSD cases. This assay is being evaluated for both newborn screening and leucocyte based assays for the diagnosis of LSD.

P42. Multiplexing Current and Emerging Enzymatic Assays for Pompe, Mucopolysaccharidosis Type I, Biotinidase Deficiency and Galactosemia Disorders on a Digital Microfluidic Cartridge

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Newborn screening (NBS) for Pompe and Mucopolysaccharidosis Type I (MPS I) disorders has been recently added to the United States Newborn Recommended Uniform Screening Panel, and there is an increasing level of global interest to implement NBS for these disorders. NBS for biotinidase deficiency and galactosemia is already performed by most public health programs. We modified our existing digital microfluidic cartridge to multiplex fluorimetric assays for acid-alpha glucosidase (GAA), acid alpha-L-iduronidase (IDUA), galactose-1-phosphate uridyltransferase (GALT) and biotinidase (BIOT) measurement. These enzymatic assays screen for Pompe, MPS I, galactosemia and biotinidase deficiencies, respectively. The assays are performed from a single dried blood spot punch using an established and validated digital microfluidic system that is currently in use in a state laboratory to screen for Pompe, Fabry, Gaucher and MPS I disorders. Each digital microfluidic cartridge contains 48 liquid input reservoirs and can run all four assays in less than 4 h using 75 nanoliter droplets of samples and reagents. The multiplexed assay presented here will enable NBS laboratories to flexibly add MPS I and Pompe to their screening panels without displacing current assays for galactosemia and biotinidase deficiency. The assays for Pompe, MPS I and biotinidase generate a fluorimetric signal using 4-methylumbilliferone, while the galactosemia assay is based on the NADPH fluorescent readout from a 3-step enzyme cascade. We will present preliminary data generated using dried blood spot specimens to highlight the potential of our multiplexed assay to combine existing and emerging enzyme assays for high throughput screening of 4 enzymatic disorders in a newborn screening laboratory.

P43. Results of Prototype Automated Creatine Kinase Muscle Isozyme Immunoassay for Potential Duchenne Muscular Dystrophy Identification

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Duchenne muscular dystrophy (DMD) causes progressive muscle degeneration and premature death affecting 1 in 3600–6000 live male births. DMD is caused by a dysfunctional dystrophin gene located in the X-chromosome. In DMD cases, the breakdown of skeletal muscle cells releases intracellular creatine kinase muscle isozyme (CK-MM) to the circulation, making it possible to screen for DMD with this biomarker.

Our objective was to evaluate the analytical performance of a prototype automated immunoassay for measuring CK-MM from dried blood spots (DBS), which is currently under development for the GSP® system from PerkinElmer.

Using a panel of DBS samples prepared from human whole blood, some spiked with purified human CK-MM, we characterized the precision, analytical limits and measuring range of the prototype assay under development. We also measured a panel of 259 presumed healthy samples and 10 affected DBS samples.

The median CK-MM concentrations of the neonatal samples and DMD affected samples were 130 μg/L (range: 29 to 804 μg/L) and 5560 μg/L (range: 1220 to 9920 μg/L), respectively. Thus the highest unaffected sample was lower than the lowest DMD affected sample. The assay could detect CK-MM down to the <10 μg/L level, so none of the neonatal samples tested were too low to measure. Three of the DMD affected samples were above the highest calibrator of the assay (8000 μg/L) and thus technically above the measuring range. The precision of the assay was acceptable within the measuring range.

The results suggest that the GSP® CK-MM assay under development has a sufficient measuring range to span the relevant range of affected and unaffected neonatal samples. There was a complete separation between the distributions of the normal and DMD affected samples in this study.

P44. Sources of Variation in Dried Blood Spots (DBS) of Prototype Automated Creatine Kinase Muscle Isozyme (CK-MM) Immunoassay

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Duchenne muscular dystrophy (DMD) causes progressive muscle degeneration and premature death affecting 1 in 3600–6000 live male births. DMD is caused by a dysfunctional dystrophin gene located in the X-chromosome. In DMD cases, the progressive breakdown of skeletal muscle cells releases intracellular creatine kinase muscle isozyme (CK-MM) to the circulation, making it possible to screen for DMD with this biomarker.

Our objective was to study the stability of CK-MM and sources of variation in DBS samples of a prototype automated immunoassay, which is currently under development for the GSP® system from PerkinElmer to measure CK-MM from dried blood spots.

Sources of variation were characterized by using a panel of DBS and liquid samples prepared from human whole blood and spiked with purified human CK-MM. We also studied the stability of CK-MM in DBS samples stored at different temperatures and humidity.

Punches from the edges of DBS showed higher variation than the punches from the middle of the DBS spot. These results suggest that CK-MM analyte might not be evenly distributed in DBS. Humidity and temperature had a significant effect on the stability of CK-MM in DBS samples. For long-term storage, DBS samples should be stored frozen to ensure the stability of CK-MM.

P45. Newborn Screening for Critical Congenital Heart Diseases (CCHD) in a Remote County of Shanghai, China

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Critical congenital heart diseases (CCHD) are severe and life-threatening diseases requiring surgical or catheter intervention within first year of life. Recently, pulse oximetry (POxS) was adopted for newborn screening of CCHD. A community-based newborn CCHD screening program was developed for Chongming Island, which is a remote county located in Shanghai, China. The birthing facilities on the Island are located about 90 km (~2 h traffic time) from the city referral medical centers and without any pediatric cardiologist on the Island.

Two birthing facilities were participated in this program between August 2014 and December 2015. At the age of 24–36 h, POxS tests were performed. A result decision chart and a CCHD Screening Assistant App <cchd.pmf.tw> were provided to assist result decision at bedside. If the newborn failed the screening test, after clinical examination the newborn was referred for an echocardiography by a local ultrasonographer immediately. The echocardiographs were reviewed by a pediatric cardiologist of the referral medical center via free WeChat App remotely.

Of 3303 live births on the island during the period, 98.2% underwent POxS. Five (0.15%) newborns had failed the screening test. All the screen failed cases were referred and confirm diagnosed before 48 h after birth. Four of them were confirmed as CCHD, 3 IAA and 1 single ventricle, one of them had diagnoses solely attributable to the CCHD screening. The other referred case was diagnosed with pulmonary dysfunction.

This efficient and effective community-based newborn CCHD screening program in Chongming Island successfully integrated screening and referral systems which provided a scheme for implementation of the newborn CCHD screening program in remote area without local pediatric cardiologist in China.

P46. Global Update of Critical Congenital Heart Disease Newborn Screening Using Pulse Oximetry

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Background: Congenital heart disease (CHD) is the most prevalent birth defect in newborns and is the leading cause of death prior to one year of age. Screening asymptomatic newborns for critical congenital heart disease (CCHD) has become the standard of care in several developed countries. National and multi-center pilot projects are well underway and will add to our ability to refine existing protocols. In the United States, an estimated 98% of births or more are currently being screened for CCHD.

Methods: Congenital heart disease advocacy groups, investigators in screening for CCHD, and international health organizations have been meeting with health care providers and government officials on a country by country basis. Countries that are implementing or have pilot projects have been identified to track global implementation.

Results: Norway, Switzerland, the United States, Poland, Ireland, and Sri Lanka have recommendations to screen at the national level. Europe is well poised for implementation with surgical and catheterization interventions for CCHD available. In Africa, Asia, South and Central America, individual countries are in the early stages of organization.

Conclusions: Screening for CCHD is spreading across the globe. Early recognition has the ability to improve care in countries providing CHD treatment and prepare parents for adverse events in countries where care is not accessible. Impact of screening in regions with less access to intervention may see the greatest impact on lives saved through the identification of secondary targets such as non-CCHD, pulmonary, and infectious pathology.

P47. NGS in Argininosuccinic Aciduria Detects a Mutation (D145G) Which Drives Alternative Splicing of ASL: A Case Report Study

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Background: Argininosuccinic aciduria (ASAuria; OMIM 207900) is a rare autosomal recessive heterogeneous urea cycle disorder, which leads to the accumulation of argininosuccinic acid in the blood and urine. We aimed to perform genetic test to the patient and help clinician to diagnose precisely.

Case presentation: In this study, we use next generation sequencing (NGS) and exon trapping to analysis the family members. We identified compound heterozygous mutations of the argininosuccinate lyase (ASL) gene in a Chinese Han ASAuria patient. The c.434A>G (p.(D145G)) mutation in exon 5 was shown by exon trapping to select for the formation of an alternative transcript deleted for exon 5. The c.1366C>T (p.(R456W)) mutation had been previously reported in an Italian patient.

Conclusions: This is the first report of a missense mutation driving alternative splicing which results in the loss of exon 5 in ASAuria. This study also demonstrates the value of NGS in the identification of mutations and molecular diagnosis for ASAuria families.

Keywords: Argininosuccinic aciduria, Next generation sequencing, Exon trapping, Alternative splicing, Molecular diagnosis

P48. Neonatal Screening: A Comparative-Historical Perspective

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After the first regional initiatives to screen newborns for PKU in the UK and US in the 1960s, several European countries set up screening programmes. Though the number of disorders screened for gradually increased, countries differed in their choices for specific screening strategies and the number of disorders screened for: from 1 to 29 (Loeber et al. 2012). For instance, in case of Congenital Hypothyrodism, the Netherlands followed Canada and several US states in the strategy used to detect both primary and central forms of CH, while most European countries concentrated on the primary forms. The Netherlands was one of the few countries to introduce screening for Congenital Adrenal Hyperplasia (in 2000). The reasons for this variety may range from health care priorities and budgets to practical considerations, organisational constraints or, perhaps, chance. Pollitt (2006) suggested differences may be related to professional backgrounds. For the US the influence of patient organisations and commercial parties has been mentioned (Paul & Brosco, 2013). For the Netherlands we did not find evidence that these latter forces have played a major role.

In our poster presentation we highlight our research on the expansion of the Dutch programme after 1974 (Loeber & Van El 2014). In addition we present an initiative to stimulate historical-comparative research on the different choices regarding the expansion of neonatal screening in Europe.

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P49. Parents' Perspectives of Medical Screening for Autism: Would They Say "Yes" to the Test?

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Background: Current diagnosis of autism spectrum disorder (ASD) is based upon behavioral and observational evaluations typically performed around age 3. Children and their families may benefit from early identification of ASD through participation in Early Intervention programs. Research suggests that a laboratory-based blood test for ASD may soon be available.

Purpose: To explore parents' perspectives of a hypothetical screening test for ASD for their child and the implications of a positive result.

Methods: Anonymous surveys were administered in two pediatric offices. The survey questions focused on parents' views of a laboratory-based ASD screening test; the possibility of equivocal screen results; acceptable test turn-around times; test costs; and child/family participation in ASD research.

Results: The majority 44/65 (68%) of respondents wanted a laboratory-based blood test for ASD. Parents with a low tolerance for ambiguity were favorable toward this type of screening. Most approved of testing as early as possible rather than wait until age 3, regardless of symptoms. We found that 75% reported if their child tested positive they would be glad to have their child benefit from Early Interventions.

Conclusion: The emerging trends in our analysis show that parents have positive attitudes toward a laboratory-based test that could indicate a risk of developing ASD before age of 3. The possibility of equivocal results and potential distress from positive results test were not major factors in parents' decisions for testing. Furthering our understanding of parent responses to new screening tests can inform our approaches to counseling and patient care delivery.

P50. Obtaining Consent for Newborn Screening

Veronica Wiley, Rosie Junek, Crystyna Smith, Tiffany Wotton, Won Tae Kim and Jake Berry

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Newborn blood spot screening has been performed in the NSW Newborn Screening Programme, Australia for 50 years. Newborn screening which is publically funded and controlled within the public health sector is offered to all newborns rather than being mandatory. Information, as multi lingual pamphlet, video and web data, on the newborn screening process is provided to families at antenatal visits, prior to delivery and again after delivery.

Until recently, enrolment for screening was considered informed dissent. Families who refuse to have newborn screening even after further discussion with a clinician and/or the staff of the programme provide information on reasons for refusal. In 2014 the process of consenting was modified to include details on the newborn screening card to be completed by the mother. Consent was requested to: perform screening; store samples beyond 2 years (the period identified as good laboratory practice); and allow de-identified research on residual samples.

Since the implementation over 158,000 cards have been received. Of these 76 families have refused collection of a sample for tests being screened, most commonly citing religious reasons. A further 26 families refused long term storage; and 28 refused de-identified research.

Obtaining signed consent did not impact the newborn screening programme in New South Wales, Australia

P51. Communicating with Parents; Technology May Help Parents Engage with Newborn Screening Results

James Bonham, Louise Moody and Lou Atkinson

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The advantages of newborn screening (NBS) are well-documented; however research also demonstrates the potential negative impact of positive screening results on family relationships, parental depression and ongoing relationships with health care professionals. The communication and support provided at first contact and during confirmatory testing is thought to affect this impact on the family.

A recent study to explore patients experience approached 30 screen positive families (18 true positive and 12 false positive) to explore their experience. The results show that the way in which the parents were approached and the quality of the information provided were influential in their response and is likely to affect the long term impact on the family.

As a result of this work a patient support group and health professionals in the UK are working together to design and evaluate an App that may be offered to women during pregnancy. The content will provide information about newborn screening and context specific information in the event of a positive newborn screening result.

P52. Development and Evaluation of Multiplex Assay of TSH, FT4 and 17-OHP by Xmap Technology for Newborn Screening

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Background: In newborn screening, simultaneous measurement system can reduce the amount of time taken to punch samples and to dispense reagents and the requirement for the amount of blood to be used. We developed multiplex assay for TSH, FT4, 17-OHP eluted from one dried blood spot punch simultaneously.

Methods: A multiplex assay for TSH, FT4, 17-OHP based on the Luminex xMAP technology was developed as the Triplex Neo TSH, FT4, 17-OHP Kit in newborn screening for congenital hypothyroidism and congenital adrenal hyperplasia. The evaluation was performed in three newborn screening laboratories in Japan. The MAGPIX Dx System (Luminex Corp.) was used as the simultaneous fluorimetry device.

Results: For the Triplex Neo TSH, FT4, 17-OHP Kit, limit of quantification was 1.0 mIU/L for TSH, 0.2 ng/dL for FT4 and 1.0 ng/mL for1 17-OHP. The measurement precision were as follow; intraassay CVs were less than 10% and interassay CVs were less than 15%. Good correlation was shown with comparing the ELISA method, and the identical distribution of TSH, FT4 and 17-OHP were shown in over 3000 newborn samples.

Conclusion: Our multiplex assay for TSH, FT4 and 17-OHP can largely reduce not only the punching of the DBS but also the man power that is necessary for measurement operation in comparison with the ELISA method. Furthermore, the detection of both thyroidal and central CH became simple and easy by simultaneous measurement of TSH and FT4, and we could improve efficiency and the cost-benefit in the CH screening. We consider that this multiplex assay system is suitable for routine use in newborn screening laboratory.

P53. *First Report of Validation Test with GSP in China*

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Background: In 2014, more than 15,000,000 babies have taken newborn screening in China. The huge number of samples requires a full automatic screening system to improve efficiency and ensure quality. In this study, TSH, Phe and 17α-OHP (with the largest number of samples in routine screening), were tested with GSP in China for the first time.

Methods: The following tests were carried out with GSP: (1) Evaluate the accuracy and precision of the method by running the kit controls at different concentration levels (two levels for TSH and Phe, three levels for 17α-OHP) repeatedly; (2) Evaluate the correlation and the total coincidence rate (including positive and negative percent agreement) between GSP kit and Ani Labsystems kit by running the clinical confirmed samples in DBS in parallel with these two methods. The clinical confirmed samples include 966 negative and 75 positive samples for TSH, 1471 negative and 84 positive samples for Phe, 1447 negative and 96 positive samples for 17α-OHP.

Results: (1) The performance of GSP is as follows: for TSH, within-lot CV is 5.6% and 6.1%, between-lot CV is 7.2% and 8.1%, and accuracy is 7.27% and 0.44%; for Phe, within-lot CV is 5.6% and 5.4%, between-lot CV is 11% and 13.3%, and accuracy is 7.52% and 8.47%; for 17α-OHP, within-lot CV is 5.2%, 4.9% and 6.4%, between-lot CV is 7.8%, 7.8% and 9.4%, and accuracy is 7.82%, 0.24% and 1.7%; (2) Highly correlated between two methods, correlation coefficient of TSH, PKU and 17α-OHP is 0.97, 0.99 and 0.94; The total coincidence rate is 96.9% (1009/1041), 99.16% (1542/1555), and 98.3% (1517/1543), respectively between two methods.

Conclusion: GSP has a good analytical performance, also with a good correlation with the product which is another routine screening system in China.

P54. Fully Automated System for PKU/GAL/MSUD/BIO Using 384 Well Microplates

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Newborn screening lab is in a consistent evolution process if considered the new technologies, new parameters and solutions. We present in this study the evolution of fully automated systems for newborn screening labs using 384 well microplates for quantification of phenylalanine, total galactose, leucine/isoleucine and analysis of enzymatic activity for biotinidase. The study evaluated approximately 5.000 samples from a routine newborn screening lab. The results demonstrate the gains obtained by the lab compared with the same equipment using the 96 well microplate format. We have increased the processing capacity with the same equipment. The time process was reduced for 3 h to run the same number of samples, more robust movement process reducing the number of steps in 75%, the consumables were reduced in 80%, use of transfer/filtering microplates were eliminated, sample size was reduced from 3.00 mm to 2.1 mm and we generate much less residual substances/components, resulting in a considerable improvement of sustainable process. The evaluation of the results in terms of each assay is presented in graphs comparing 96 with 384 well microplates. Nimbus NeoLISA 384 shows the evolutionary process to bring continuous improvement to the newborn screening labs and programs for screening of PKU, GAL, MSUD and biotinidase.

P55. Dried Blood Spot-Based Analysis of the Cyanoethylvaline-Adduct of Hemoglobin for Smoking Assessment

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Smoking during pregnancy can affect the unborn baby in multiple ways, since cigarette smoke contains more than 4000 chemicals. Efficient ways to screen if a pregnant woman came in contact with cigarette smoke or smoked herself, are needed. Here, we present a new dried blood spot (DBS)-based method to screen for exposure to acrylonitrile, one of the chemicals in cigarette smoke. When acrylonitrile enters the bloodstream, it binds covalently to hemoglobin, thus forming an adduct, the N-terminal cyanoethylvaline-adduct (CEV). In contrast to nicotine and cotinine, which only have a short half-life, this CEV adduct remains present for months, offering a wide window of detection. The use of DBS offers many benefits since it is a minimally invasive sampling approach for the patient, which is especially relevant for newborns.

Existing methods to screen for the CEV adduct are based on a modified Edman degradation. This procedure allows specific detachment and isolation of N-substituted N-terminal valine as a thiohydantoin derivative. However, these methods are lengthy (about 16 h of derivatization) and require several milliliters of blood. In our method, we implemented microwave-based on-spot derivatization of DBS and further optimized the degradation. In the optimized procedure fluorescein isothiocyanate is added onto a 6 mm DBS punch and derivatization is performed for 15 min in the microwave at 300 W. Further sample clean-up is done by solid phase extraction. For the measurement of this adduct an LC-MS/MS/MS method was developed. The method was already successfully applied on a genuine smoker sample. Following validation, this methodology will be applied on DBS from mothers and newborns, to evaluate the potential of this novel approach to assess smoking behavior.

P56. Fully Automated Direct Extraction and Analysis of Dried Blood Spots for the Determination of Four Anti-Epileptic Drugs and Two Active Metabolites

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Dosage adjustment of anti-epileptic drugs (AEDs) by therapeutic drug monitoring is very useful, especially for children. Considering the benefits of dried blood spots (DBS), this matrix could be an alternative to conventional venous sampling for this purpose. Since manual punching and off-line extraction slow down DBS analysis, an automated direct extraction and analysis of DBS can be advantageous. Therefore, we developed a method for the determination and quantification of four anti-epileptic drugs and two active metabolites (valproic acid, carbamazepine, phenobarbital, phenytoin, oxcarbazepine and carbamazepine-10,11-epoxide), using a prototype device for on-line extraction of DBS samples followed by automated on-line solid phase extraction (on-line DBS-SPE) prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Optimization of the LC-MS/MS method included the comparison of different mobile phases in combination with different HPLC-columns, the testing of different gradient elution programs, the adaption of source- and compound dependent parameters and the incorporation of deuterium labeled internal standards. Furthermore, different clamps, extraction solvents and SPE cartridges were combined during the method development of the on-line DBS-SPE system. A next step is to validate the developed method based on U.S. FDA and European Medicines Agency (EMA) guidelines for bioanalytical method validation. This will encompass the evaluation of selectivity, carry-over, matrix effect, stability, recovery, hematocrit-effect, volume-effect and volcano-effect. After completing the validation, the method will be applied on patient samples originating from developing countries, to demonstrate the benefits of DBS sampling of AEDs in pediatrics in developing countries.

P57. Assessing Performances and Usability of the NS2400 Automated Platform from Labsystems Diagnostics

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Newborn Screening in most countries is highly centralized with daily sample loads of 300 to over 2000 in a few cases. Therefore totally automated systems are highly desirable. The main advantage of these systems is less hands-on-time for the laboratory personnel, better traceability of the working steps, and less danger of mistakes and false screening results. The NS2400 from Labsystems Diagnostics combines total automation by replacing exactly the technician processed without altering the product design. The same kit reagents can be used as manually and/or be integrated in the NS2400 platform ensuring continuity of measurement in case of downtime. The automate's modules can also be used as standalone providing further flexibility of use. We tested the system thoroughly with all so far available tests (TSH, 17-OHP, IRT, Biotinidase, total Galactose, G-6-PDH, and PKU) and comparison with routine tests are reported.

P58. A Simple Method to Overcome the "Floating Disc Problem" Using the GALT-Assay on the Perkinelmer GSP

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The Perkin Elmer Genetic Screening Processor (GSP) TM is a fully automated system for the processing of immunoassays for TSH, 17-OHP, IRT, biotinidase, and total T4, as well as enzymatic assays for total galactose and galactose-1-phosphate uridyltransferase (GALT) from dried blood spots (DBS). The system however, has one drawback: it cannot transfer samples from one microtiter plate to another one. While this is not a problem with immunoassays, it makes enzymatic assays more problematic, because dried blot spots have to remain in the wells and can cause significant signal quenching, thereby reducing sensitivity, or they can increase fluorescence intensity, when the extracted spots are floating on the surface of the reacting mixture. The later can cause false negative results, when GALT activity is measured for galactosaemia screening. To overcome this problem, the GSP includes

an additional measurement step to check for floating disks, leading to prevention of the affected measurements. However, this causes a secondary problem in this totally closed system. We detected floating disk signals in approx. 0.5% of all screening as well as quality control samples, which had to be repeated. In some cases even a second sample had to be requested, because floating disks were detected again in the 2nd, 3rd, and 4th repeat assays. To overcome this problem, we developed a method employing a second-tier measurement on a victor fluorescence reader. Using this second-tier measurement made all repeat measurements unnecessary.

P59. Blood Spot Sampling Cot Cards in a Tertiary Neonatal Unit; A Prospective Cross-Sectional Study

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Background: This prospective cross-sectional study was conducted at a tertiary neonatal unit (NNU).

Aims: Newborn blood spot screening is mandatory in Scotland and is powered to detect five diseases (ref. 1), each with significant morbidity and mortality if untreated. Neonatal admission to NNU disrupts the routine screening process, with opportunities for screening missed. There is no known detrimental impact upon patient care, however this lapse does not adhere to our NNU standards. The extent of the problem was assessed by a pilot project which quantified the rates of bloodspot completion on the NNU. Upon finding that our recording was poorly consistent with actual sampling, a further investigation highlighted that timeliness of the samples required improvement (MW). The results of the initial analysis were disseminated to staff groups during audit meetings (January 2015).

Intervention: A new cot card system was created. The labels contained: Baby name, DOB; Days 1/5/28: Sample due date, Sample taken, Electronic documentation; Consent.

Methods: Data was collected in 2 periods: Data1 (pre-imp): 05/08/2015–29/09/2015 (8 weeks). Creation of cot cards for all admissions, updating of current blood spot status; 02/10/2015–05/10/2015; Medical/Nursing Safety Briefs explain the cards. Troubleshooting period: 05/10/2015–13/10/2015. Data 2 (post-imp): 14/10/2015–08/12/2015 (8 weeks).

Results: The effect of introducing cot cards was assessed with a period of post-implementation data collection which matched the pre-imp period, to reduce reporting bias. The results of analysis are outstanding. The anticipated benefit is that there will be a greater number of blood spot samples sent on the recommended date, possibly with a reduction in the number of unnecessary repeat samples. The anticipated impact is that it will allow earlier detection of preventable diseases.

P60. Mass Spectrometer Detector Saturation Compromising Performance in Newborn Bloodspot Screening

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The analysis of blood spot amino acids such as leucine and phenylalanine using mass spectrometry can be compromised due to detector saturation. Using an AB Sciex API 4000 mass spectrometer effects were observed at leucine concentrations as low as 300 µmol/L (significantly below the 600 µmol/L UK referral value for maple syrup urine disease (MSUD)).

Small changes in performance of the mass spectrometer also led to variation in the point at which detector saturation occurred as did matrix effects (particularly External Quality Assessment (EQA) and Quality Control (QC) specimens).

QC and EQA performance was examined against signal intensity and a re-assay protocol was developed based on signal intensity rather than concentration.

Reduced volume injection was compared against dilution as a method for extending the linear range. However, reducing the injection volume down to 2 μL proved insufficient to remove saturation effects in a significant number of cases and so a stepwise protocol (incorporating reduced volume followed by dilution where necessary) was adopted.

The use of more sensitive instruments for newborn blood spot screening can lead to compromises when analysing amino acids at higher concentrations. Analysts need to be aware of detector saturation characteristics which can vary with instrument type, performance and sample matrix. Failure to recognise and correct for detector saturation could result in missing cases of MSUD and inappropriate treatment for patients with phenylketonuria.

P61. *Performance Comparison of Two Filterpapers Used for Whole Blood Collection in Neonatal Screening*

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Neonatal screening for 17 illnesses is available for all babies born in The Netherlands. It is conducted in one of five screening laboratories using whole blood obtained from a heel prick spotted onto a filter paper at least 72 h postpartum. In 2016 the heel prick card filter paper, Whatman#903 (W#903) will replace PerkinElmer#226 (PE#226). We present the results of a study comparing the two filter papers in the Dutch screening laboratories.

Experimental: Adult whole blood collected in lithium heparin vacutainers was pipetted (50 μL) onto both filter papers and dried at ambient temperature. The dried blood spots (DBS) were punched, prepared and run as routine samples for newborn screening. Newborn screening analytes (35) were assessed in duplicate at five laboratories, on three separate days across a one-week period.

Results: Performance characteristics (DBS absorption time and diameter), accuracy, precision and analyte stability for 15 selected analytes, with concentrations in a quantifiable range and reflecting the range of analytes and analytical methods, have been assessed. Good concordance between filter papers and the five screening laboratories was found. With the exception of 17α-OH-progesterone (OHP), analyte concentrations were comparable (%CV < 25); OHP exhibited greater variability (%CV < 34, range 1.0–2.0 nmol/L). Precision for the majority of analytes was acceptable (%CV < 10). No statistically significant difference was found between measured analyte concentrations for the duration of the study; for all laboratories the PE#226/W#903 ratio for the mean measured concentrations of each analyte was 1.01, 1.04 and 0.95 on day 1, 2, and 8 respectively (mean 1.0; range 0.9–1.1). Our data show the performance of PE#226 and W#903 filter papers is equivalent.

P62. *Stability of Newborn Screening Markers in Dried-Blood Spot (DBS): The Innovative Imagene Solution*

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In France, 5 newborn diseases are screened on DBS. Residual DBS are stored refrigerated at least for one year. The time window for reliable retrospective analyses is depending on the stability of the markers. Imagene company has developed an original technology for DNA and RNA preservation at room temperature (RT) in laser-sealed capsules ensuring a totally anhydrous and anoxic atmosphere. To evaluate this new storage procedure for DBS, we studied the stability of 32 markers. Sets of DBS

samples (anonymous blood, Neobase control high, PerkinElmer, Turku) were stored at either −20 °C or 4 °C at low humidity, and at RT in Imagene capsules (Study A). Markers levels were measured every month during the first year and then at 18 and 24 months. We also performed two accelerated degradation studies whereby DBS samples were stored in high-humidity environment at RT (Study B) or 37 °C (Study C) and in Imagene capsules at RT, for one month (ten measures). In study A, after six months, all the markers concentrations remained stable in the 3 conditions with the exception of hemoglobin S (HbS) which was seen to be unstable at 4 °C, stable at −20 °C and exhibited minor degradation in Imagene capsule. In studies B and C, after one month of exposition to highly humid air at RT or 37 °C, the concentration of carnitine (C0) increased respectively by about 62% and 41% while acylcarnitines C2, C3, C4, C6 lost more than 50% of their initial levels. The concentration of glutamic acid increased more than 70%. Arginine, methionine, HbS and SCID markers lost more than 50% of their initial levels. Glycine, ornithine, phenylalanine, tyrosine, immunoreactive trypsinogen, thyroid-stimulating hormone and thyroxine lost more than 40% in high-humidity at 37 °C. On the contrary, when protected in Imagene capsules, these markers exhibited no change in concentration.

P63. Evaluation of the Effect of Glue Contamination on Analysis of Newborn Screening Samples

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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. As far as we are aware, this is the first study to evaluate this potential contamination on NBS analyses. Experimental: EBF #1003 glue (50 μL, three concentrations) was blotted onto Whatman#903 dried blood collection cards (W#903) using a plastic template and dried at ambient temperature. Adult whole blood was collected in a lithium heparin vacutainer, pipetted (50 μL) onto the glue blots and dried at ambient temperature. In addition, blank glue blots (i.e., no blood) and dried blood spots (DBS) in the absence of glue were prepared. The DBS and blank samples were punched, prepared and run in duplicate as routine samples for NBS. Two wells immediately after samples containing glue contained only analytical solutions (AS), i.e., extraction and analysis buffers.

Results: DBS absorption time and diameter, interference, carryover and precision for 16 selected analytes have been assessed. DBS absorption time, diameter and shape are equivalent for DBS prepared in the absence and presence of undiluted glue. When EBF #1003 was diluted prior to use, DBS absorption time increased and DBS were non-uniform. Interference and carryover was not identified for the analytes, methods and instruments evaluated. Precision for DBS prepared in the absence and presence of glue was equivalent and acceptable (%CV < 22) for all analytes with concentrations in the quantifiable range. Our data show that in the unlikely event of contamination of W#903 with EBF #1003 there is no effect on the measured concentration of analytes.

P64. Investigation of the Stability of Analytes Used for UK Newborn BLOODSPOT Screening by Length of Time between Sample Collection and Receipt within the SE Thames NBS Population

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Introduction: The UK Newborn Screening Programme currently advises that samples received more than 14 days after collection are not suitable for analysis. A review of samples received in our laboratory during 2015 identified 157 cards which were not suitable for analysis due to delay in transit.

This study reviewed all cards received in 2015 to investigate the relationship between transit time and population percentiles for the analytes measured under the UK screening programme.

Method: A report was created within the laboratory IT system that gathered all the results of screening cards received in 2015. Cards were removed from the data if they had been rejected for any other reason apart from being older than 14 days, *n* = 57,963. The 50th, 90th and 99th percentiles were calculated using Microsoft Excel for each of the analytes measured dependent on number of days between date of collection and date of receipt.

Results: The 50th percentile for methionine showed a decrease of 23% from 22 μmol/L to 17 μmol/L between cards collected and received same day and cards that were received 5 days after collection. The 90th percentile had a decrease of 20% from 30 μmol/L to 24 μmol/L. The 99th percentile had a decrease of 59% from 78 μmol/L to 32 μmol/L. There was no significant change in concentration with increasing transit time for the other analytes.

Conclusion: The decrease in methionine identified in this study suggests the current 14 day acceptance criteria may lead to false negative results for bloodspot homocystinuria screening.

P65. Investigation on Whether the Analytes Used for UK Newborn Bloodspot Screening Are Stable at Room Temperature Based on the Screening Programme 14 Day Rejection Criteria.

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Introduction: January 2015 saw the UK implement screening by MS/MS for five additional disorders: homocystinuria, GA1, IVA and MSUD. The majority of cards are sent through the mail system which leads to an average delay of 3 days between collection and receipt by the laboratory. Currently the UK Newborn Screening programme advises cards are suitable for testing up to 14 days after collection. This study investigated the stability of the analytes for these disorders in cards stored at room temperature for 14 days.

Method: Ten healthy volunteers each provided a single 5 mL lithium heparin whole blood sample (anonymised). The blood samples were spotted onto filter paper to provide 28 spots per volunteer. The blood spots were left to dry and then packaged into 28 bags containing ten spots, one from each volunteer. Over the next 28 days one bag per day was transferred from storage at room temperature to −20 °C. The spots were then thawed and analysed simultaneously for the five analytes following laboratory standard protocol. Analytes concentrations changes over time were evaluated by linear regression.

Results: Between day 3 and 28 there was a significant decrease in the methionine concentration ($p < 0.01$). The average concentration dropped from 20.31 μmol/L to 17.93 μmol/L. All other analytes for the conditions screened did not show significant differences in concentrations over the 28 days.

Conclusion: This small study showed significant changes in concentration of methionine over a period of 28 days at room temperature. This could mean a change in outcome dependent on age of sample for homocystinuria with a false negative card reported. Though as UK screening laboratories reject samples that are older than 14 days the risks of a false negative due to age of sample is minimal.

P66. Six Years of Neonatal Screening of Inherited Metabolic Disorders in the Czech Republic

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Background: Early diagnosis of inherited metabolic disorders (IMD) enables early treatment, and improves prognosis, and quality of patients' life. We present the results of 6 years of expanded neonatal screening program targeted at 10 IMDs in the Czech Republic.

Methods: Amino acids and acylcarnitines were extracted from dried blood spot samples and analyzed by tandem mass spectrometry.

Results: Between October 2009 and December 2015 we analyzed samples from 688,243 newborns. We detected 194 patients with subsequently confirmed IMD yielding a detection rate of 1:3550. The positive predictive value was 27% and false positive rate 0.07%, we are aware of one missed patient with intermittent MSUD. For the most frequent diseases (PKU/HPA 1:5250; MCADD 1:20,900 and LCHADD 1:76,500) frequency of pathological alleles and its geographical distribution in regions were evaluated.

Conclusion: Although performance of expanded neonatal screening met the criteria recommended by the Region4Genetics project further improvement is desirable. Our laboratory algorithms are still being optimized in order to reduce number of false positive cases. After carrying out a pilot project the national authorities are preparing expansion from 10 to 15 IMDs by adding citrullinemia type I, argininemia, CBS/methylenetetrahydrofolate reductase deficiency and biotinidase deficiency. After expansion the neonatal screening program in the Czech Republic will be able to detect a total of 37 IMDs.

P67. *Performance Monitoring in Newborn Screening—A Co-Ordinated National Approach*

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In January 2015, the NHS newborn blood spot screening program was expanded to include four additional disorders detected by MS/MS. Nationally agreed screening protocols were adopted with specified analytical and clinical cut-off values (COV) so harmonisation between labs was important. Most labs use in-house MS/MS methods with quantitation of results based on isotope dilution alone.

To assist with performance monitoring and provide assurance of the efficacy of the screening programme, the national programme co-ordinating centre commissioned a team to collect and analyse population data and results from common IQC material prepared at three levels from all 14 national screening labs. This was collated each month and discussed alongside EQA data at quarterly national meetings attended by all screening labs.

Approximately 600,000 babies were screened in 2015. The population centiles of 8 analytes were determined, by individual lab and collectively. The interlab variation of the 90th centile ranged from 27%–59%. QC data was used to determine the measurement uncertainty (MU) associated with each analyte. MU ranged from $\pm20\%$ to $\pm36\%$. The Horwitz ratio indicated the analytical performance of leucine and phenylalanine was sub optimal.

The COV was generally well removed from the 90th centile and the concentrations seen in affected patients are many times greater again. While the screening programme is unlikely to produce a false negative result, there is real potential to produce false positives, causing unnecessary stress and anxiety to families. In view of this harmonisation of laboratory results need to be improved. This will prove challenging due to a lack of certified reference materials and calibrators and the financial implications associated with commercial kits.

P68. *Rate of Abnormal Newborn Screens Suggestive of Amino Acid Breakdown Disorder Differs by Regional Neonatal Intensive Care Nursery and Formulation of Parenteral Nutrition*

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Background: Parenteral nutrition (PN) may lead to elevated amino acid (AA) levels and an abnormal newborn screen. Neonatal intensive care units (NICUs) in New Zealand use 2 different AA solutions. Primene® was designed to achieve a plasma AA profile similar to cord blood and TrophAmine, which has a two-fold greater concentration of methionine, to achieve plasma AA levels of healthy 30 day-old breastfed term babies.

Aim: To determine if the rate of abnormal AA levels on newborn screening tests differs according to NICU and the AA solution used for PN.

Method: National data on NICU screening tests were reviewed over a 5 year period (2010–2015). The routine protocol includes a 1st sample at 48 h, plus repeat tests for low birth weight infants (<1500 g at 2 weeks, < 1000 g at 2 + 4 weeks). AAs were measured by tandem mass spectrometry. The rate of abnormal AA screens was calculated for individual NICU and grouped according to AA solution.

Results: Of 15,633 NICU 1st samples, 609 (3.9%) had abnormal AA screens (459 with elevated methionine). Abnormal AA screens were found in 64/3888 (1.6%) of 2nd samples and 18/1325 (1.4%) of 3rd samples. The rate of abnormal AA screens for individual NICUs ranged from 0.8%–10.7% on 1st samples, 0.1%–3.2% on 2nd samples and 0%–3% on 3rd samples. Overall, the rate of abnormal AA screens was 10-fold greater amongst samples from NICUs that used TrophAmine as compared with Primene®. No clinically unsuspected inherited AA disorders were detected.

Conclusion: False positive screening tests suggestive of AA disorders were especially common amongst NICUs that used TrophAmine, an AA solution with relatively high methionine concentration. Whilst there are sound clinical arguments behind the use of such solutions, screening programmes should consider tailoring AA cut-offs to nutrition. More detailed feeding histories would be informative.

P69. Pilot Study for Evaluation of 21 Additional Metabolic Disorders for the German Newborn Screening Panel

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Background: The German newborn screening panel currently includes fourteen target disorders. Recent improvements in diagnostic and therapeutic options suggest an extension of the newborn screening panel.

Patients/Methods: From summer 2016 onwards the Heidelberg newborn screening centre will be performing a pilot study to evaluate newborn screening for 21 additional metabolic disorders for the German newborn screening panel, including e.g., remethylation disorders, classical homocystinuria, vitamin B12 deficiency, tyrosinemia type I, methylmalonic and propionic aciduria and urea cycle disorders. The designated diagnostic algorithms including second tier strategies will be presented. It will be prospectively evaluated whether the extension of the newborn screening panel fulfils the criteria for a population based screening program, especially concerning technical feasibility, process quality, and medical benefit. As an example, the clinical histories of two patients diagnosed symptomatically with one of the new target disorders (combined cobalamin deficiency and severe maternal vitamin B12 deficiency) are presented and their newborn screening results retrospectively evaluated.

Conclusion: We expect that a considerable number of children will benefit from screening for the additional target disorders in the course of the pilot study and in case of a future comprehensive extension of the newborn screening panel for Germany.

P70. Evaluating Diseases on the Newborn Screening Panel—PKU as an Example

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In Canada, there are no national newborn screening recommendations that provide guidance to provincial programs regarding which diseases should be on screening panels, for evaluating potential new screening targets, or for re-evaluating existing targets. In Ontario, a formal process was developed for periodic review of individual diseases on the Newborn Screening Ontario (NSO) panel to determine whether changes need to be made. The robustness of the review process was trialed with phenylketonuria (PKU), selected as the longest standing disease on the newborn screening panel, and one whose screening process was not expected to change as a result of the review process. A review form was completed consisting of four sections: the condition (prevalence, clinical spectrum targeted by screening), the test (positive predictive value, other possible screening modalities), the treatment (availability and effectiveness), and societal considerations (cost of screening, non-targeted outcomes). Both program data and existing literature were used to populate the form. The review process identified the following issues: (1) The need for more precise disease definitions; (2) The need for quality indicator benchmarks; and (3) The need for long-term follow-up data. While no changes to the actual screening of PKU were recommended, the review highlighted that periodic review of newborn screening processes and outcomes, even screening for a disease as "straightforward" as PKU, can identify system issues that require further coordination and discussion. Addressing such issues will improve the NSO program and allow for a better evaluation of this and other diseases in the future.

P71. Pilot Study on Expanded Newborn Screening in Slovenia: Preliminary Results

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Slovenia currently screens newborns only for phenylketonuria and congenital hypothyroidism. Last year a pilot study of expanded newborn screening for inborn errors of metabolism using tandem mass spectrometry started. 10,000 dried blood spots from newborns were analysed retrospectively for the following disorders: MCAD (medium-chain acyl-CoA dehydrogenase deficiency), GA 1 (glutaric acidemia type 2), GA 2 (glutaric acidemia type 2), 3-MCC (3-methylcrotonyl-CoA carboxylase deficiency), MSUD (maple syrup urine disease), VLCAD (very long-chain acyl-CoA dehydrogenase deficiency), LCHAD (long-chain 3-hydroxyacyl-CoA dehydrogenase deficiecy), IVA (isovaleric acidemia), PA (propionic acidemia)/MMA (methylmalonic acidemia), CUD (carnitine uptake disorder), CPT 1 (carnitine palmitoyl transferase 1 deficiency), CPT 2 (carnitine palmitoyl transferase 2 deficiency). We also included PKU (phenylketonuria), so we could compare our results with the results of the current method for phenylketonuria screening (fluorimetric detection of phenylalanine).

Results: 5 cases were identified so far; one VLCAD deficiency (confirmed with sequencing of ACADVL, enzymatic activity analysis, palmitate loading test), three 3-MCC deficiencies (confirmed with analysis of organic acid analysis in urine) and one GA 1 (confirmed with enzymatic activity analysis). The study also detected two known patients with PKU. Based on the preliminary results from the pilot study the cumulative incidence of inborn errors of metabolism (1:1429) is high in Slovenia. We are currently doing follow-up tests on selected newborns with the highest disease possibility to set the cut-off values for the chosen disorders.

P72. Newborn Screening in Zhejiang Province China

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Objective: To control and decrease the incidence of birth defects and increase the population quality by screening for inherited metabolic disease in newborn infants to identify and treat affected babies in the early stage.

Method: Congenital hypothyroidism (CH), Phenylketonuria (PKU), amnio acid disorders, fatty acid oxidation disorders and organic aciduria disorders are identified from newborn heel prick blood samples dried on filter paper. The babies are 3–7 days old born in the confinement units of Zhejiang Province.

Result: Since the newborn screening(NBS) started from September 1999, NBS has covered all the 495 confinement units in Zhejiang province and the present uptake rate is 99.54% in 2015. Among the 7,822,687 newborns screened in the past 17 years, 4791 CH, 362 PKU/BH4D are identified with the incidence of 1/1633 and 1/21,610 respectively. There are 952 cases of transient hypothyroidism. Among the 1,440,771 newborns screened by Tandem Mass Spectrometry since 2008, 284 cases of genetic metabolic disorders are identified including 124 cases of amino acid disorders, 73 cases of organic aciduria disorders and 87 cases of fatty acid oxidation disorders with the total incidence of 1/5073. PKU/BH4D is the most frequent disorder in amino acid disorder with 60.48% (75/124), while methylmalonic acidemia (MMA) and 3-methylcrotonyl-coenzyme A carboxylase deficiency (MCCD) in organic aciduria disorders with 36.99% (27/73) and 26.03% (19/73) and primary carnitine deficiency (PCD) in fatty acid oxidation disorders with 71.26% (62/87).

Conclusion: More metabolic disorders can be identified by Tandem Mass Spectrometry and the incidence of birth defect can be controlled effectively.

P73. Performance Metrics of 5 Years of Newborn Screening in the Czech Republic

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Background: The nationwide newborn screening (NS) in the Czech Republic is currently performed for congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), cystic fibrosis (CF), phenylketonuria/hyperphenylalaninemia (PKU/HPA), leucinosis (MSUD), glutaric aciduria type I (GA I), isovaleric aciduria (IVA), medium chain acyl-CoA, long chain 3-hydroxyacyl-CoA and very long chain acyl-CoA dehydrogenase deficiency (MCADD, LCHADD and VLCADD), carnitine palmitoyltransferase I and II deficiency (CPTD I and II) and carnitine-acylcarnitine translocase deficiency (CACTD). The aim is to evaluate NS data from period I/2010–XII/2014.

Methods: A total of 551,023 newborns were screened by use of fluoroimmunoassay for thyrotropin, 17-hydroxyprogesterone and immunoreactive trypsinogen (IRT). The CFTR gene (32, later 50 mutations) was analysed in 5790 (1.05%) blood spots with the highest IRT levels. The spectrum of metabolites was analysed by tandem mass spectrometry.

Results: A total of 485 captured patients represent cumulative detection rate 1:1,136. In CH was found screening prevalence (P) 1:2599 and positive predictive value (PPV) 0.58; in CAH P = 1:13,776 and PPV = 0.02; in CF P = 1:6,888 and PPV 0.13; in PKU/HPA P = 1:5,298 and PPV = 0.37; in MCADD P = 1:19,679 and PPV = 0.72; in LCHADD P = 1:55,102 and PPV = 0.77; in VLCADD P = 1:137,756 and PPV 0.06; in GA I and IVA P = 1:183,674 with PPV = 0.12 and 0.04; in MSUD P = 1:551,023 and PPV = 0.01. No case of CPTD I and CPTDII/CACTD was detected.

Conclusion: NS is an effective approach for presymptomatic detection of serious rare diseases. Further optimization, the additional analytical tiers to reduce false positivity and expansion of screened disorders, is needed.

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P74. Inborn Errors of Metabolic Screening Laboratory in Qatar: A Successful Journey

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Background: Neonatal screening is the most important preventive public health program of the 21st century. It is implemented in the majority of the developed countries. The screening of newborns in Qatar was previously very limited. Because of the limited screen, all newborn screening specimens were sent abroad for detection of metabolic disorders. This process delayed the reporting and interpretation of results and delayed treatment of affected babies. The Department of Laboratory Medicine and Pathology (DLMP) moved forward to establish a comprehensive newborn screen laboratory at HMC—Qatar.

Method: With the purchase of instrumentation and recruitment of personnel. Personnel underwent training in recognized world class newborn screening laboratories including University Clinics Heidelberg and Mayo Clinic. The Metabolic Screening Laboratory systematically developed, validated, and introduced newborn screening tests on dried blood spots DBS.

Results and discussion: In 2015, the metabolic laboratory performed newborn screening for amino acids/acylcarnitines using 104 primary markers and ratios for over 55 disorders. A total of 25,880 newborn babies were screened compared to 20,506 in 2013. The false positive rate was 0.19% compared to 0.16% in 2013 with 0% false negatives for both 2013 and 2015. The true positive rate was 0.090% or 1/1125 compared to 0.098% or 1/1025 in 2013. Over the past 36 months, 100% of the external proficiency test results were acceptable for all tests and specimens. Aminoacidopathies account for 52% of the disorders (Homocystinuria ($n = 4$), CitrullinemiaII ($n = 2$), PKU ($n = 6$)), Fatty Acid Oxidation Disorders (MCAD ($n = 3$), CUD ($n = 2$), SCAD ($n = 2$)) 26%; Organic acidemias (3MCC ($n = 3$)) 13% and 4.3% CPS/OTC.

P75. Newborn Screening in Buenos Aires Province—Argentina. 24 Years of Experience

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Background: Newborn Screening (NBS) in Buenos Aires Province (BAP) was implemented by Fundacion Bioquimica Argentina (FBA) together with Children's Hospital "Sor Maria Ludovica" (HSML) following a sequential process: 1991 Phenylketonuria (PKU), 1992 Congenital Hypothyroidism (CH), 1995 Cystic Fibrosis (CF) and Galactosemia (GAL), 1997 Congenital Adrenal Hyperplasia (CAH) and Biotinidase Deficiency (BD), and 2001 Maple Syrup Urine Disease (MSUD). Firstly, all the

diseases were screened by request, without a program organization. Just in 1995, the "Diagnostic and Treatment of Congenital Diseases Program" (Prodytec) was implemented by the BAP Ministry of Health, becoming the first organized and centralized program implemented in Argentina. In a first stage, it covered only for PKU and CH, giving fulfillment to the law in force. In December 2008, a new law was enacted expanding the panel to CF, GAL, CAH, BD and MSUD, being mandatory in practice from 2010 onwards.

Objective: To present the results of 24 years of NBS experience in BAP.

Methods: The functional organization of the program includes screening at the FBA NBS Laboratory, and diagnosis, treatment and follow-up at the HSML, giving free of charge coverage to all newborns (NB) born in public hospitals since July 2010. NBS was made using in-house fluorometric methods for PKU, GAL and MSUD, an in-house colorimetric method for BD, and AutoDELFIA for CH, CF and CAH.

Results and Conclusions: Until December 2014, 3,634,953 NB were screened for PKU (PKU: 1:26,926, HPA: 1:23,604); 3627,152 for CH (1:2169); 1,762,642 for CF (1:8314); 1,427,775 for GAL (GAL-T Def.: 1:75,146); 1,317,842 for CAH (Salt Wasting CAH: 1:14,324); 1,054,292 for BD (Profound BD: 1:131,787) and 830,040 for MSUD (1:118,577). The program coverage in 2014 reached 97.2% for public hospitals and 62.8% for the total of BAP (~32% of NB remaining are tested in other laboratories). Currently, Prodytec makes an important contribution to NBS in Argentina testing around 210.000/year, which represent the 27% of NB born in the country.

P76. External Quality Assurance Program for Neonatal Screening of Glucose-6-Phosphate Dehydrogenase Deficiency

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The national neonatal screening program for G6PD deficiency was started in 1987 and 1996 in Taiwan and Philippines, respectively. To assess the reliability and assure the quality of the screening and confirmatory tests, external quality assurance (EQA) programs for G6PD tests were developed. The QC materials were prepared from human blood with human G6PD. Periodically (2–3 month), EQA survey samples were sent to participating laboratories. The test results were submitted online and the summary reports were published on the website. <g6pd.qap.tw>.

Currently, 50 screening laboratories (including 4 reagent manufacturers) from 15 countries and 44 referral laboratories in Taiwan and Philippines are participating in the EQA program. Since 1999, 99 EQA surveys for screening test were performed, 202 (10.2%) unsatisfactory reports were found. The unsatisfactory results were mainly caused by inappropriate cut-offs. Between 1988 and 2015, 192 EQA surveys were sent to referral laboratories in Taiwan, 306 (8.6%) unsatisfactory reports were found. Since 2007, the error rates have been decreased to less than 4%. Inter-laboratory C.V. for the quantitative test has reached < 10% in recent years. The long term (7 years) intra-laboratory precision (C.V.) of the referral laboratories in Taiwan were 6.6% (1.6%~20.5%). From 2009 to 2015, 35 EQA surveys have been carried out for the newly established network of referral laboratories in Philippines, 81 (18.8%) unsatisfactory reports were found. Inter-laboratory C.V. in Philippines were between 6.6% and 25.0%, which is lower than those found in other EQA programs (e.g., CAP, RCPA) for G6PD quantitative test. These EQA programs have been useful to improve the G6PD tests quality, and might be a reference to adjust the cut-offs for the screening test.

P77. Effect of Introducing National Criteria on the Newborn Blood Spot Avoidable Repeat Rate

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Introduction: For many years, the West Midlands Newborn Screening laboratory has focussed on training of Midwives to improve the quality of newborn screening blood spot samples. The avoidable repeat rate (ARR) has been consistent over the years (~4.0%), yet still above the national standard of 2.0%.

The NHS newborn blood spot screening programme launched a new blood spot quality initiative in April 2015 to improve blood spot quality and standardise rejection/acceptance criteria across the newborn screening labs in the UK. We report the ARR before and after the national criteria and investigate areas for further improvement.

Method: The calculated ARR from all samples received from February 2015 to February 2016 were reviewed. ARR was categorised into insufficient, unsuitable, too soon after blood transfusion, too young 4 days.

Results: The ARR decreased to 2.0% with the introduction of the new criteria. In September, a new multipunch was implemented, the Panthera-Puncher 9, which uses 'intelligent' punching. The use of the punch initially increased the ARR but then the ARR decreased as the importance of improving blood spot quality was communicated to healthcare professionals.

Conclusion: Acceptance/rejection criteria clearly has an impact on the ARR, as does the use of 'intelligent' punching. Consistent monthly reports to screening leads and QA teams emphasising the importance of blood spot quality has reduced the ARR. Continued education and training is required to reinforce the importance of blood spot quality in newborn screening.

P78. Introducing the Dutch Neonatal Screening Programme in Overseas Dutch Caribbean Netherlands. Organisational and Logistic Challenges Overcome

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Introduction: As of October 2010 the status of the overseas parts of the Dutch Kingdom changed: they now were special municipalities within the Kingdom of the Netherlands and as such, they were entitled to the same public health care, including neonatal screening (requested by the Island-authorities in 2011). Upon ministerial decision a feasibility study was performed. Prerequisites for this feasibility study were: the screening should be identical to the screening in continental Netherlands, preferably, existing infra-structure should be used, and clinical referral should preferably be organized locally.

Methods: Here, we report on the compilation of the feasibility study (finalized April 2013), and the actual implementation of the screening programme. We describe the educational (with special reference to language), logistic (transporting the heelprick samples to a Dutch laboratory) and analytical challenges, as well as the first results of the screening programme.

Results: The feasibility study was conducted between July 2012 and April 2013 and covered all of the above project aims. Based on the report [i], both the authorities of Bonaire, St Eustatius and Saba sanctioned the implementation of NBS screening.

The programme was first piloted on the Island of Bonaire and went live 1 January 2015. To date 169 NBS samples were received; findings were limited to 4 cases of HbS carriership. Using the Bonaire-experience, a pilot was also started on the much smaller islands of St Eustatius and Saba. Full implementation was realized 1 October 2015.

Discussion: In summary, in a two year period, in a project with motivated efforts from all participants at two sides of the Atlantic Ocean, we succeeded in transferring the Dutch heelprick programme to the Dutch Caribbean Islands, under the same quality control constraints as those of the longstanding continental programme.

i.   Dekkers, E.H.B.M.; Abbink, F. *Feasibility Study for the Implementation of the Neonatal Heel Prick Screening in the Dutch Caribbean*; RIVM Report No. 120007001/2013; 2013.

P79. Public Involvement in Policy Making for Newborn Screening: Goals, Definitions, Mechanisms, Levels, and Evaluation

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Technological developments have influenced newborn bloodspot screening (NBS) in the past and will probably influence the purpose of programs now and in the future. A shift in the purpose of NBS has implications for the public. However, the public is not structurally involved in NBS policy decisions. In light of current discussions about expanding NBS via Next Generation Sequencing (NGS), an approach to involve the public is needed for meaningful and successful public health policy development.

To summarize different key elements for public involvement in policy making for NBS, a rapid review of literature was performed. Search terms included: newborn, screening, public involvement, genetics, and decision making. Furthermore, a snowball method was applied where the references of resultant key articles were checked for additional relevant articles.

Five key topics were summarized from literature: the goals of public involvement, how to define "public", which mechanisms exist for public involvement, on what levels it can prove to be valuable, and relevant indicators to evaluate the outcome. Most literature focused on public involvement in general, and the literature discussing NBS illustrated that the involvement is often through patient representatives, takes place ad hoc, and focuses on informing rather than deliberating.

Public involvement does not take place in a structured or transparent manner in current NBS policy making. It is relevant to shape such involvement and develop a model to include relevant views from the public, and also debate whether expanding NBS is the right mechanism to implement possibilities from NGS.

P80. A Retrospective Audit in Decline Rates in the South East Thames Screening Laboratory

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Introduction: Before a child is screened the parents are allowed to make an informed decision about whether or not to consent to testing. In some cases, the parents choose to decline screening. The aim of this audit was to determine the decline rate over a selected period and to observe any trends in the decline samples that we receive.

Method: Data was collected from the LIMS between 1/5/13 and 31/7/15 and categorised by specified time periods and location to investigate any trends in the data.

Results: 209 declines were received during this period, representing 0.15% of the workload. Results separated by quarter showed no significant increase in any one quarter. However data showed a gradual increase in the number of declines between November 2014 and July 2015. In the final quarter the majority of declines came from London PCTs of which 32% were babies that were born outside of the UK or reported to have testing completed elsewhere. Over the whole period, 38 of the 209 declines gave "child born abroad" or "testing done previously" as a reason.

Conclusions: The decline rate for SE Thames Newborn Screening laboratory is very low. However, the number of declines in the last six months has risen from previous quarters. 38 (19%) of the declines were due to babies being born outside of the UK who have previously had screening elsewhere. This is therefore unlikely to be something the laboratory can influence and represents the large "moving in" population that we see in London today. The number of true declines is therefore lower (158), representing 81% of the total.

Future Work: To put these results in perspective, a comparison is currently undergoing between the SE Thames data and decline data from the two other London screening labs, to identify whether other regions see a similar trend.

P81. New Progress and Challenges of Newborn Screening in China

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In the early 1980s, newborn screening started preliminarily from some hospitals in the developed areas in China, but only a small number of newborns were screened. Until 2003, the Chinese government has intensified the administrative management on newborn screening and has enacted the Technological Guideline of Newborn Screening. Phenylketonuria (PKU) and congenital hypothyroidism (CH) were firstly recommended as the universal screened conditions. Since then, newborn screening has been developing regularly and the number of newborns screened has been increasing yearly. In 2008, the national newborn screening rate was approximately 51%, of which the rate in some developed provinces even reached to 100%. In 2009, our government has enacted the Regulation on Newborn Screening Management, which expanded the disorders screened to three ones: PKU, CH, and congenital hearing loss. In 2010, the Newborn Screening Subsidizing Program in the Poor Areas was implemented in the Middle and Western areas. During 2010 to 2014, the government has invested a total of 230.3 million RMB into 364 poor counties for newborns screened. Through this program, the national newborn screening rate for the Middle and Western areas has increased from 42% in 2009 to 87% in 2014. Meanwhile, the medical cost of PKU has been included in the New Rural Cooperative Medical System in some provinces, which insured the affected infants. Moreover, more local governments have provided the free screening on every newborn. In 2014, the national newborn screening rate has increased to 91%.

Although newborn screening have improved significantly in the past decade, several critical challenges remain, mainly including: (1) the limited disorders screened; (2) unable to provide every newborn with screening freely; (3) the significant geographic variation on newborn screening, with the lowest rate of 37% in some developing areas; (4) unimproved social security system, which lead to a heavy medical and rehabilitation cost for those affected children.