Newborn Screening

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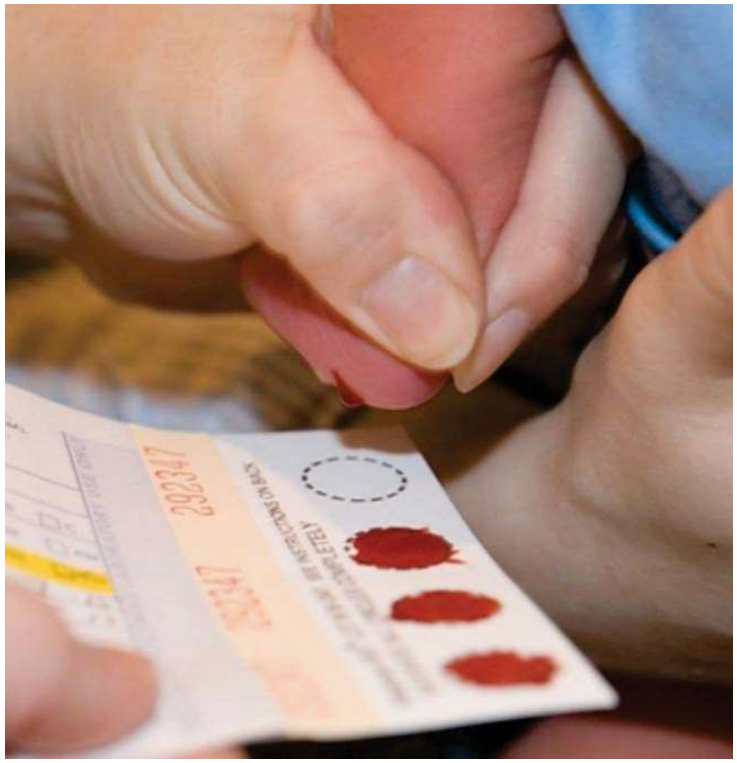
HISTORY

In the 1960s, Dr. Robert Guthrie developed a bacterial inhibition assay for the early detection of phenylketonuria (PKU)

- Classical PKU -AR inherited deficiency of the enzyme phenylalanine hydroxylase, which catalyses the conversion of phenylalanine to tyrosine
- Patients present with progressive, irreversible neurological impairment and reduced pigmentation



INCIDENCE IN SOUTH AFRICA

- Is IEM rare to justify screening?
- Hitzeroth and co-workers found 1 case PKU and 1 case tyrosinemia among 60 000 screened cases.
- Van der Watt: Type 1 glutaric acidemia 1:5000 case (highest incidence in the world)
- Prof L Mienie: Isovaleric acidemia, propionic acidemia, galactosemia, vitamin B-responsive methylmalonic acidemia, maple syrup urine disease and glutaric acidemia type I are more common

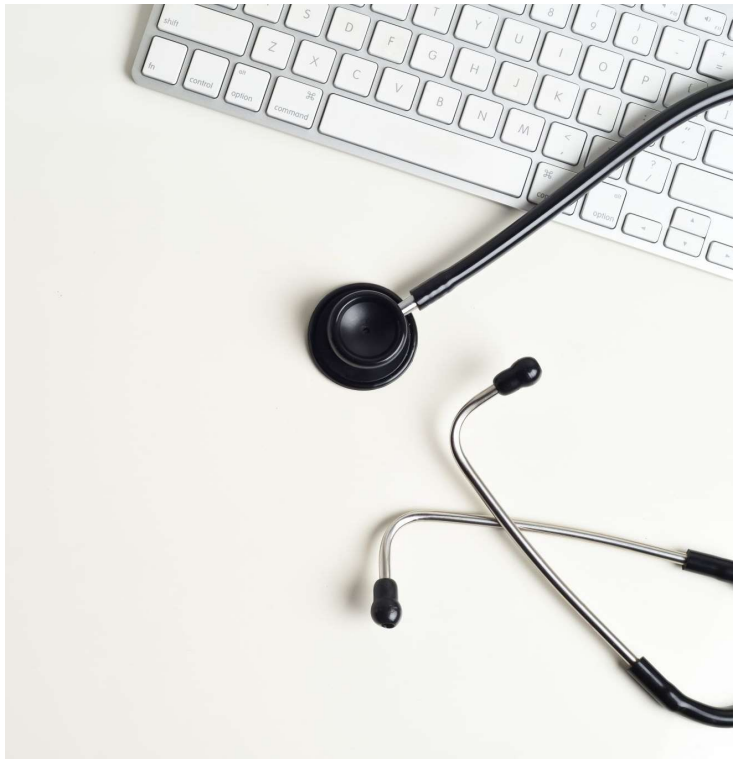
INCIDENCE IN SOUTH AFRICA

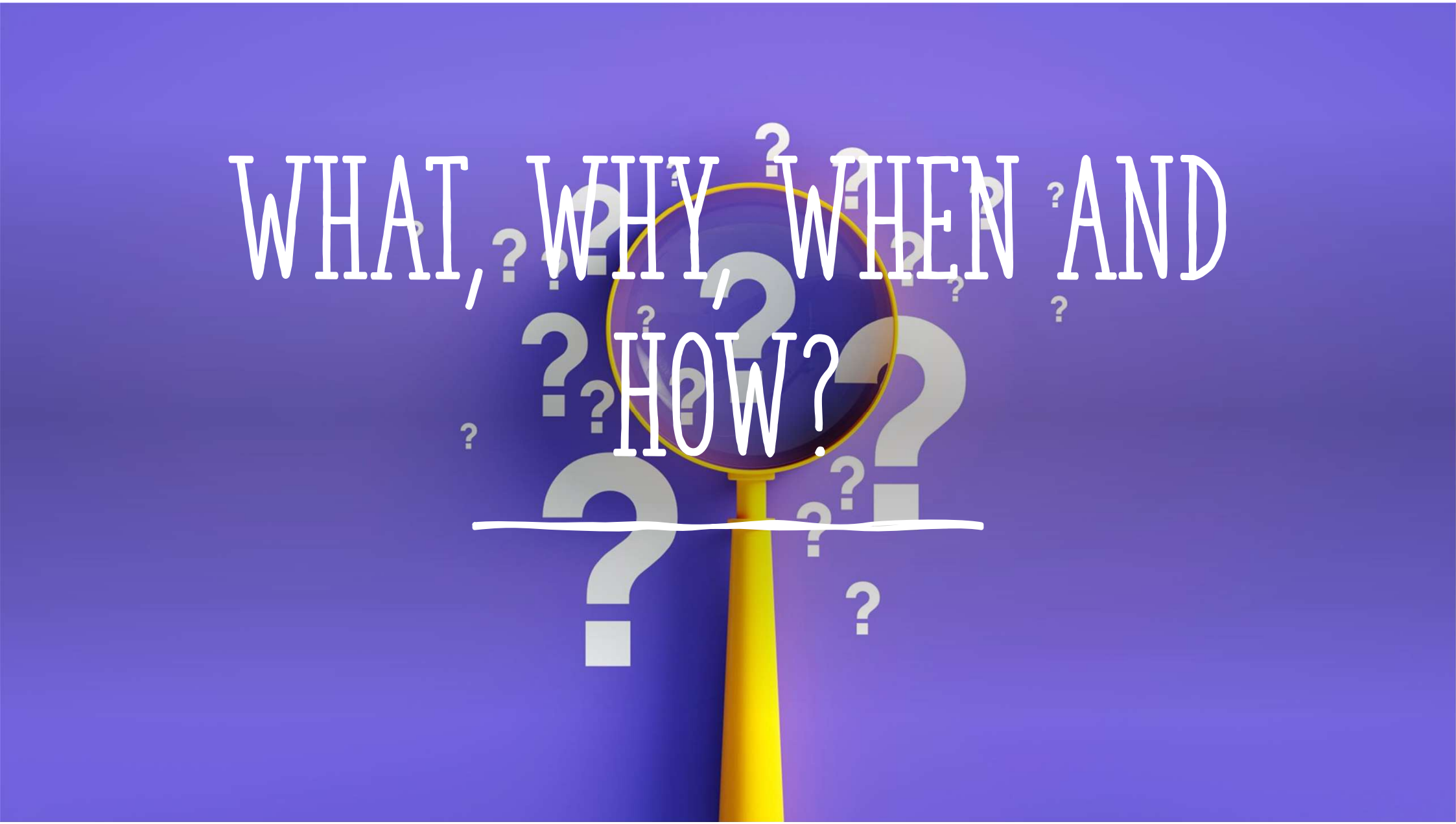
Country	2011 GDP per capita (in thousand of USD)	Newborn screening coverage (year of estimation)
Egypt	6.5	75% (2007)
China	8.3	25% (2006)
Thailand	9.4	97% (2006)
Brazil	11.8	80% (2005)
South Africa	11.0	**<1%** (2012)

CONSIDERATIONS FOR THE PRACTISING

Several obstacles face the clinician who would like to offer newborn screening to patients:

- No policy in South Africa
- Most medical insurance companies do not reimburse newborn screening (PMB)
- This lack of reimbursement creates all sorts of questions about the validity of the service in the minds of patients, who then address these questions to the clinician, who has to cope with the additional counselling workload
- Dealing with false positive screens and the associated anxiety of parents is an intricate part of newborn screening




WHAT, WHY, WHEN AND
HOW?

WHAT? CONDITIONS TO BE SCREENED

- The specific conditions screened for can vary by country and region, but they often include:
  - ***Metabolic disorders***, such as phenylketonuria (PKU) or maple syrup urine disease (MSUD)
  - ***Endocrine diso***rders, like congenital hypothyroidism
  - ***Haemoglobin disorders***: such as sickle cell disease
  - ***Cystic Fibrosis***
  - ***Severe Combined Immunodeficiency (SCID)***
  - ***Hearing loss***
  - ***Critical congenital heart defects (CCHDs)***

WHEN?

- Timing: first 24 to 48 hours after birth
- A second screen may be recommended around the 1-2 week mark to ensure accuracy, especially if the baby was born prematurely or if the initial results were inconclusive

HOW?

Blood Test (Heel Prick Test): A few drops of blood are collected from the newborn's heel onto a special filter paper.

This blood sample is then sent to a laboratory for analysis.

Hearing Test: This can be done using Automated Auditory Brainstem Response (AABR) or Otoacoustic Emissions (OAE). Both are quick and painless.

Pulse Oximetry: A non-invasive sensor is placed on the baby's skin to measure the oxygen levels in the blood, which can help detect CCHDs.

WHY?

Early Detection: The primary purpose of newborn screening is to identify conditions that can benefit from early intervention. Catching these conditions early is a proactive step that can prevent serious health problems, developmental delays, and, in some cases, death.

Preventative Care: Early treatment can significantly improve outcomes, reducing the burden on healthcare systems and families.

Public Health: It helps to prevent the spread of certain infectious diseases, collect data for research, and better understand rare conditions.

TYPES OF NBS

***1. Tandem Mass Spectrometry (MS/MS)* to** detect multiple metabolic disorders by analysing small molecules in the blood.
- **Function**: Measures amino acids, acylcarnitines, and other metabolites to identify conditions like phenylketonuria (PKU), maple syrup urine disease (MSUD), and fatty acid oxidation disorders.

2. ***Immunoassays***
- **Use**: Measures hormone levels and detects proteins or other substances in the blood.
- **Common Techniques**: Enzyme-linked Immunosorbent Assay (ELISA): This test detects hormones like thyroid-stimulating hormone (TSH) for congenital hypothyroidism.
- Radioimmunoassay (RIA): Another method for hormone detection, although less commonly used due to safety concerns associated with radioactive materials.

3. ***DNA Testing***
- **Use**: Identifies specific genetic mutations that cause certain disorders.
- **Techniques**:
-Polymerase Chain Reaction (PCR): Amplifies DNA segments to detect mutations or deletions.
Next-generation sequencing (NGS) provides detailed information about genetic sequences used for comprehensive genetic screening.

TYPES OF NBS

4. ***Fluorescence-based Assays***
- **Use**: Detects various substances in the blood using fluorescent tags.
- **Application**: Used in molecular assays to identify specific DNA sequences or measure certain metabolites' concentration.

5. ***Gas Chromatography-Mass Spectrometry (GC-MS)***
- Use: Separates and identifies volatile substances in the blood.
- **Application**: Often used to detect organic acidemias by measuring organic acids in the urine or blood.

6. ***Capillary Electrophoresis***
- **Use**: Separates molecules based on their size and charge.
- **Function**: Analyzes haemoglobins to screen for hemoglobinopathies like sickle cell disease and thalassemia.

TYPES OF NBS

7. ***High-Performance Liquid Chromatography (HPLC)***
- **Use**: Separates and measures components in a mixture.
- **Application**: Used to detect haemoglobin variants and other metabolic markers.

8. ***Colorimetric Assays***
Use: It demonstrates its versatility by measuring the concentration of chemicals in the blood through a wide range of chromatographic changes.
- **Example**: Used for the initial detection of galactosemia by measuring levels of galactose and galactose-1-phosphate

9. ***Fluorescence Polarization Immunoassay (FPIA***)
- **Use**: Measures concentrations of small molecules in blood by detecting changes in the polarization of fluorescent light.
- **Application**: Commonly used for newborn screening of congenital adrenal hyperplasia (CAH).

TYPES OF NBS

10. ***Enzyme Assays***
- Use: Measures the activity of specific enzymes in the blood.
- **Function**: Identifies enzyme deficiencies that cause metabolic disorders, such as biotinidase deficiency and lysosomal storage disorders.

11. ***Pulse Oximetry Screening***
- **Use**: Measures oxygen saturation in the blood.
- **Application**: Screens for critical congenital heart defects (CCHDs) by detecting low oxygen levels.

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TABLE 2: COMPARISON OF THE SUGGESTED CORE SCREENING PANEL OF THE AMERICAN COLLEGE OF MEDICAL GENETICS AND THE CURRENT SCREENING PANEL OF THE NORTH-WEST UNIVERSITY (NWU)

American College of Medical Genetics core panel	NWU screening programme	Clinical presentation
Amino acid disorders		
Classic phenylketonuria	Yes	Severe intellectual disability, reduced pigmentation, hypertonia and posturing
Maple syrup urine disease	Yes	Acute neurological deterioration, metabolic acidosis, maple syrup odour
Tyrosinemia type I	In development	Liver disease
Argininosuccinic aciduria	Yes	Acute neurological deterioration, hyperammonemia
Citrullinemia type I	Yes	Acute neurological deterioration, hyperammonemia
Homocystinuria	No	Initially normal, developmental delay, thromboembolic complications, osteoporosis, lens dislocation
Endocrine disorders		
Congenital adrenal hyperplasia	Yes	Dehydration, various degree of sexual ambiguity
Congenital hypothyroidism	Yes	Initially normal, failure to thrive, severe intellectual disability, enlarged fontanelles, macroglossia, constipation, dry and thin skin
Disorders of fatty acid oxidation		
Medium-chain acyl-CoA dehydrogenase deficiency	Yes	Sudden unexplained death, hypoketotic hypoglycaemia, liver disease
Trifunctional protein deficiency	Yes	Hypoketotic hypoglycaemia, cardiomyopathy
Very long-chain acyl-CoA dehydrogenase deficiency	Yes	Hypoketotic hypoglycaemia, cardiomyopathy
Carnitine uptake defect	In development	Cardiomyopathy
Long-chain L-3-Hydroxy dehydrogenase deficiency	In development	Hypoketotic hypoglycaemia, cardiomyopathy
Haemoglobinopathies		
Beta-thalassemia	No	Initially normal, failure to thrive, pallor, painful dactylitis, recurrent infections
Sickle cell anemia	No	Initially normal, severe anemia
Organic acidemias		
3-Hydroxy-3-methylglutaric aciduria	Yes	Neurological deterioration, hypoketotic hypoglycemia, metabolic acidosis
3-Methylcrotonyl-CoA carboxylase deficiency	Yes	Acute neurological deterioration, metabolic acidosis, hypoglycemia
Cobalmine responsive methylmalonic acidemia (Cbl A, B)	Yes	Acute neurological deterioration, metabolic acidosis
Glutaric acidemia type I	Yes	Acute brain injury with subsequent neurological impairment
Holocarboxylase synthase deficiency	Yes	Acute neurological deterioration, metabolic acidosis, convulsions, skin rash and alopecia
Isovaleric acidemia	Yes	Acute neurological deterioration, metabolic acidosis, sweaty feet odour
Methylmalonic acidemia (mut)	Yes	Acute neurological deterioration, metabolic acidosis
Propionic acidemia	Yes	Acute neurological deterioration, metabolic acidosis
β-Ketothiolase deficiency	Yes	Initially normal, developmental delay, episodic keto-acidosis
Other disorders		
Biotinidase deficiency	Yes	Hypotonia, developmental delay, skin rash and alopecia, convulsions
Classic galactosemia	Yes	Liver disease
Cystic fibrosis	Yes	Initially normal, chronic lung disease, failure to thrive
Critical congenital heart disease	No	Heart failure, cyanosis
Hearing loss	No[#]	Initially normal, delayed language development
Severe combined immuno-deficiencies	No[$]	Initially normal, recurrent serious infections

Testing for hearing loss is not part of the programme as offered by the NWU, but can usually be requested separately from a practising audiologist.

$ Testing for severe combined immunodeficiencies is not currently part of the NWU screening programme, but can be requested separately from Ampath.

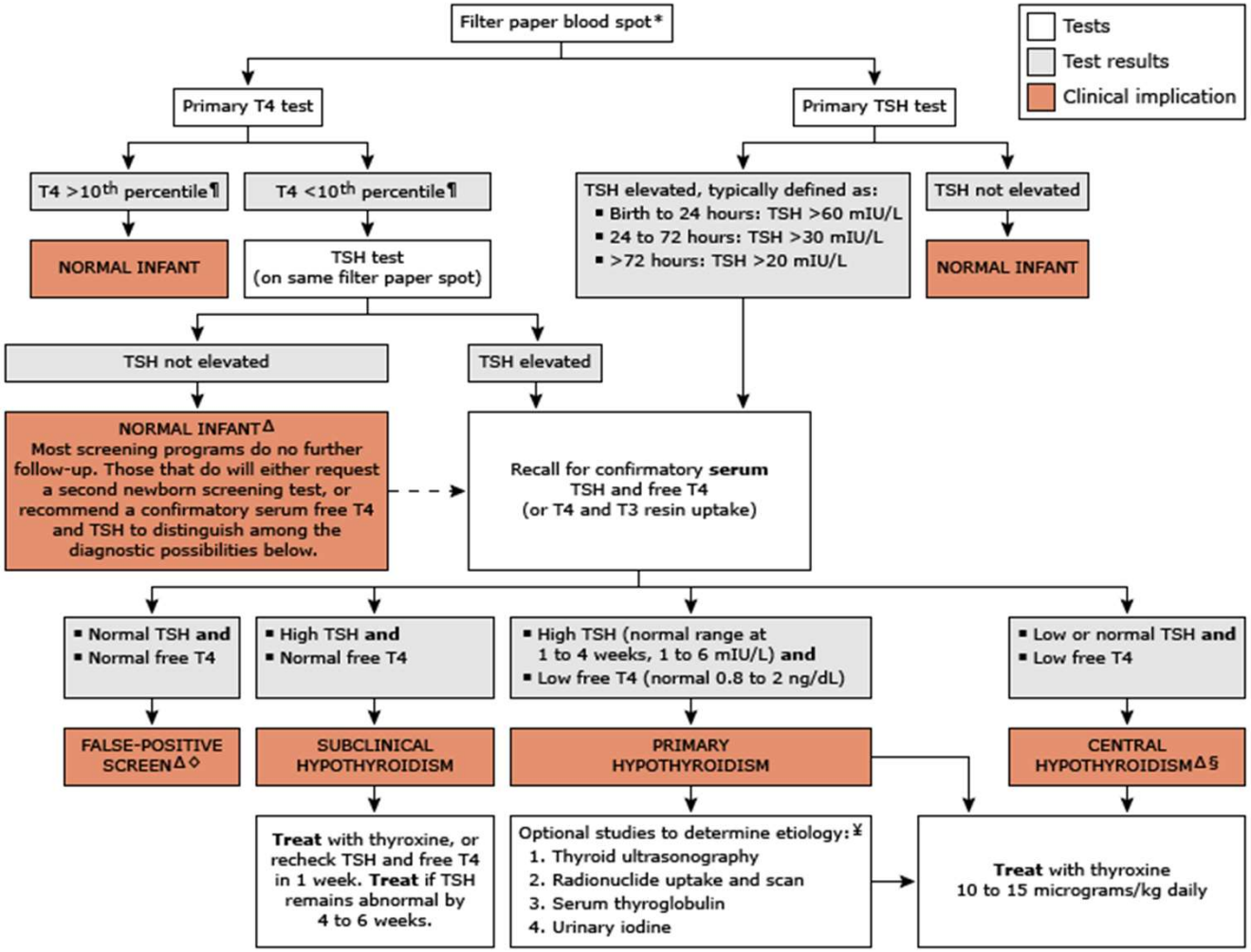
THE PAST IN SA

- In SA, community genetic services have been neglected in the last 20-30 years due to competing health priorities
- Inadequate financial and human resources and ineffective use of available infrastructure prevent an appropriate response to the growing health need
- While significant reductions in child mortality have been achieved through targeted health interventions (e.g., PMTCT for HIV, expanded immunisation programme, improved maternity care, *etc*.), improvements to the IMR and U5MR have slowed since 2011, highlighting the need to address other health issues
- In SA, as seen globally, the number and rate of CDs are estimated to be decreasing, but the proportion of CD-related deaths is increasing.
- The prioritization of CDs is not just a necessity, but a crucial step towards improving public health in SA!

CONGENITAL HYPOTHYROIDISM

- The global application of radio-immunoassay to assess thyroid function, a case was made for NBS of CH in SA in the late 1970s
- High birth prevalence (1 in 4,000 or 0.25 per 1,000 live births),
- The availability of screening and follow-up diagnostic tests with standard, low-cost (in comparison with many other IEMs) levothyroxine treatment to prevent disease progression
- Clinical onset ranges from days, weeks, or months after birth, depending on the cause and extent of thyroid dysfunction.
- By this time, intellectual deficits are irreversible regardless of subsequent thyroid replacement therapy.

Algorithm for screening and diagnosis of congenital hypothyroidism[1,2]

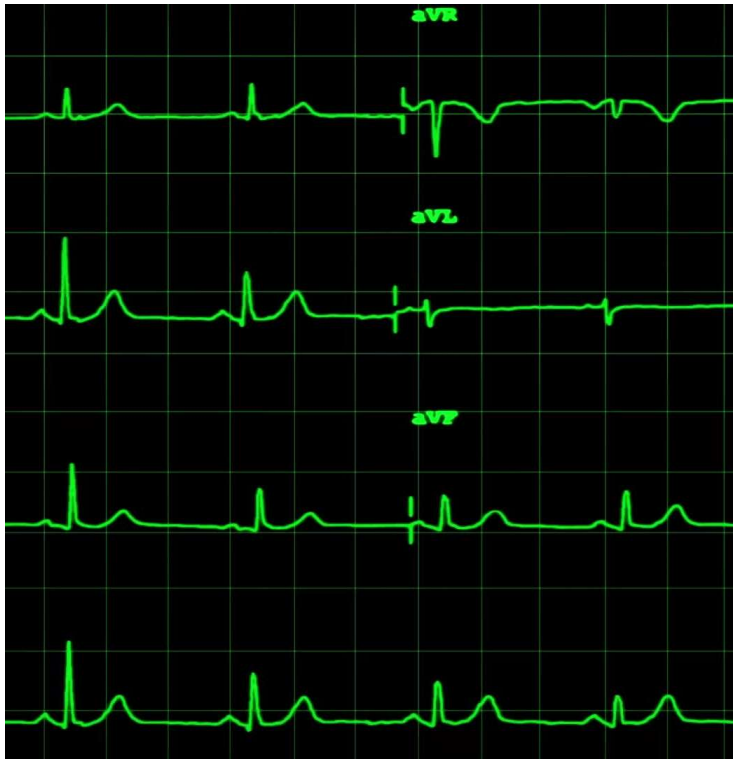


PROGNOSIS

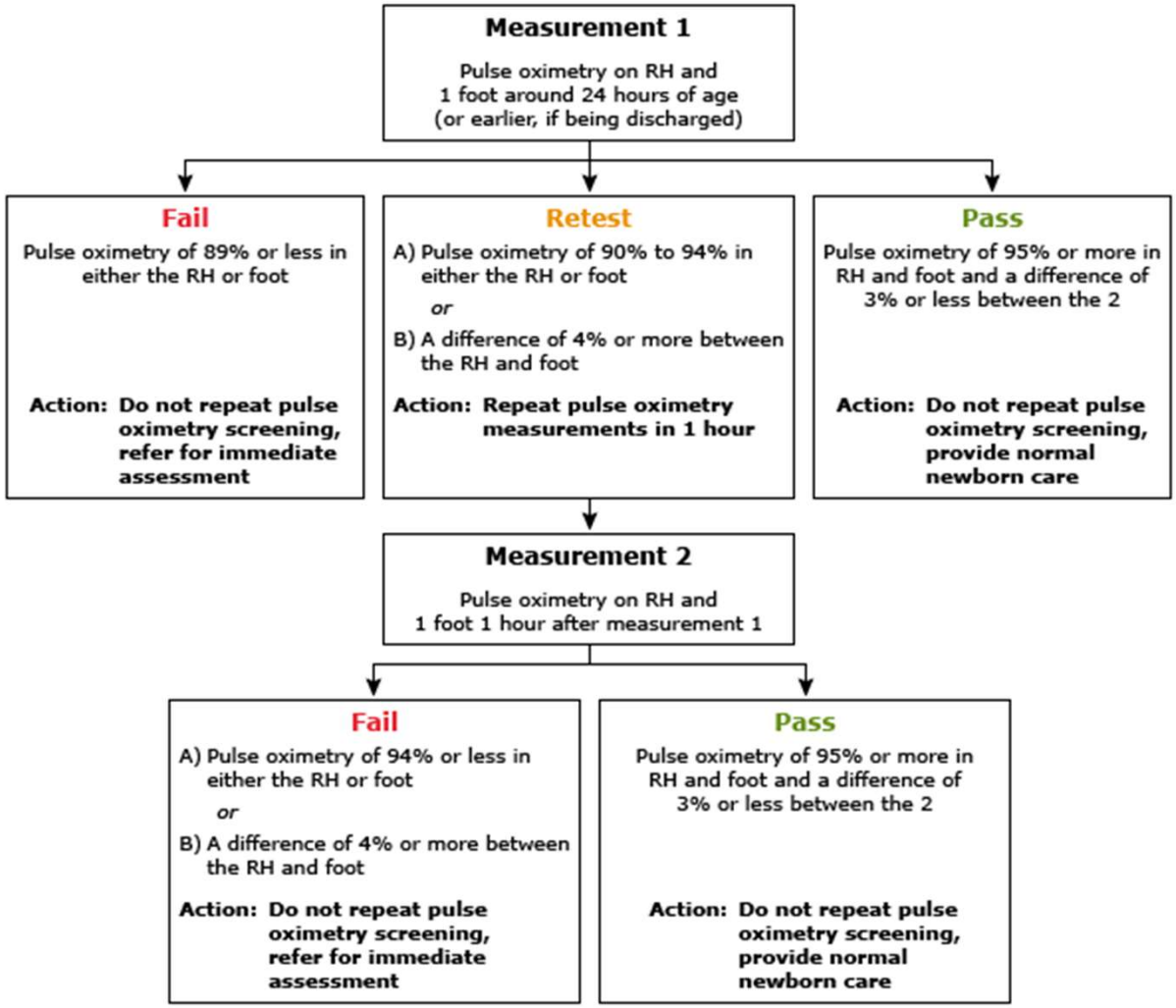
- **Neurodevelopment and functional outcomes**
- Primary hypothyroidism: Good (treatment 2-6 weeks of life and optimally first 3 years of life). IQ was similar to those of normal infants. (Long-term observational study of 76 patients with CH)
- Inadequate treatment and noncompliance: Adversely affects neurodevelopmental outcomes. One study mean IQ 87 vs 105. Another study showed improved compliance increased mean IQ score from 106 to 112
- The severity of disease: Quebac screening network lower IQ (mean IQ score 89 Vs 104 at 12 years) and impaired growth
- More severe disease associated with hearing loss- 20% sensorineural hearing loss
- Early (12-30D) vs delayed treatment- Literature review: IQ scores 15.7 points higher
- High vs low dose starting of levothyroxine- 10-15mcg/kg better IQ

PREVALENCE AND CLINICAL FEATURES OF CCHD

- 1-3/1000 births
- **Early presentation**: Shock, cyanosis and tachypnoea
- Hypoplastic left heart syndrome, Critical aortic valve stenosis, Critical coarctation of the aorta
- **Late presentation**: Feeding difficulties, poor weight gain, cyanosis, RD and excessive sweating
- HLHS, COA, IAA. AS, D-TGA, Pulmonary stenosis, TOF
- Special setting: high altitude, out-of-hospital delivery
- Cost-effectiveness



Modified algorithm for critical congenital heart disease screening with pulse oximetry



LIMITATIONS

1**. False Positives**
- Temporary Low Oxygen Levels: Newborns can sometimes display transient lower oxygen levels right after birth, unrelated to CCHDs, leading to failed screenings.
- Sensor Issues: Improper attachment or positioning of the sensor can cause inaccurate low readings.

2. **False Negatives**
-Undetectable CCHDs: Certain types of CCHDs might not cause significant hypoxemia (low blood oxygen levels) in the immediate newborn period, thus escaping detection.
- Intermittent Symptoms: Some heart defects may not cause continuous low oxygen levels and might present symptoms intermittently, leading to a normal screening during testing.

3. **Timing and Diagnostic Delay**
-Early Screening: Conducting the screening too soon after birth can lead to the non-detection of CCHDs due to the newborn's physiological adaptation to breathing air.
- Late Diagnosis: Conditions that develop or worsen after the screening period may be missed, delaying critical interventions.

4**. Technical and Operational Challenges**
- Sensor Placement: Accurate readings depend on correct sensor placement, which can be challenging with small or squirmy newborns.
- Peripheral Perfusion: Newborns with poor peripheral circulation may give inaccurate readings.
- Motion Artifacts: Movement of the newborn during screening can lead to incorrect readings.

LIMITATIONS

5**. Resource- Intensive**: Training Needs: Staff need proper training to perform the screening and interpret the results correctly.
- Equipment Availability: This requires appropriate pulse oximetry equipment, which might not be available in all medical settings, particularly in resource-limited areas.

6. **Anxiety and Stress for Parents**
- Emotional Impact: A false positive result can cause considerable anxiety and stress for parents, leading to unnecessary additional testing and hospital visits.

7. **Variability in Screening Protocols**
- Lack of Standardization: Different hospitals and regions might use varied protocols regarding the timing and method of screening, affecting the consistency of results.
- Different Thresholds: Inconsistent thresholds for what constitutes a "fail" can lead to variability in detection rates.

8. **Complementary Diagnostic Tools Needed**
- Pulse oximetry is not a Stand-Alone Test. It should be part of a broader diagnostic protocol, and relying solely on it could result in missed diagnoses.
- Need for Echocardiography: A definitive diagnosis of CCHDs typically requires echocardiography, which is more resource-intensive and not always immediately available.
- Training Needs: Staff need proper training to perform the screening and interpret the results correctly.
- Equipment Availability: Requires appropriate pulse oximetry equipment that might not be available in all medical settings, particularly in resource-limited areas.

THE FUTURE OF NBS IN SA

- A brief report on a two-day meeting held in Cape Town in February 2023, a meeting that was crucial in shaping the future of biochemical NBS for South Africa.
- The meeting addressed the importance of NBS, technology requirements, and the need for a comprehensive demonstration project for biochemical CH NBS.

Key challenges identified:

- Early newborn post-delivery discharge, technical, logistical, and infrastructure issues, as well as limited financial and human resources

CONCLUSION

- In South Africa, the value of biochemical NBS needs to be fully and urgently considered to reduce the burden of childhood mortality and morbidity.
- Little progress has been achieved to date, leaving the country far from a universal biochemical NBS programme.
- South Africa is responsible for taking the lead in expanding biochemical NBS services, with a particular focus on CH.
- Without biochemical NBS, babies affected by CH, IEMs and other rare diseases and CDs will be left behind, dying prematurely or facing a lifetime of disability.