



Section 6 of 7 - Investigation of neonatal deaths

6.1	Introduction	1055
6.2	Recommended minimal investigations for all neonatal deaths	1055
6.3	Recommended core investigations for high risk newborns	1066
6.4	Further investigations for high risk newborns at the time of birth	1066
6.4.1	Suspected congenital infection including birth after clinical chorioamnionitis and spontaneous preterm labour and delivery	1066
6.4.2	Suspected congenital abnormalities, hydropic and severely growth restricted infants	1066
6.4.3	Severe cardiorespiratory depression	1077
6.4.4	Suspected thrombophilic disorders: pre-eclampsia, fetal growth restriction	1077
6.4.5	Macrosomic infant	1077
6.4.6	Suspected genetic metabolic disorders	1098
6.4.7	Sudden unexpected neonatal death	1098
6.5	Alternative investigations where permission for autopsy is not obtained	1109
6.5.1	External examination by a perinatal/paediatric pathologist, clinical geneticists or paediatrician	1109
6.5.2	Babygram	1109
6.5.3	Magnetic Resonance Imaging (MRI)	1109
6.5.4	Clinical photographs	1110
6.5.5	Other alternatives to a full post-mortem	1110
6.6	References	111
Appendices		
Appendix 1	High risk newborn investigation checklist	11211
Appendix 2a	Screening for genetic metabolic disorders	11312
Appendix 2b	Components of the genetic autopsy for investigation of metabolic disorders	11413

SECTION 6 INVESTIGATION OF NEONATAL DEATHS

6.1 Introduction

Neonatal deaths can result from disorders of the neonate, placenta or mother. The majority of neonatal deaths are due to major congenital abnormalities and complications of preterm birth. Due to the presence of a wide range of aetiological, clinical and geographic circumstances across the spectrum of neonatal deaths, the nature of investigations undertaken following death may vary widely. For example, the investigation of the sudden collapse and death of a newborn receiving standard hospital postnatal care will require a very different investigative approach to that of an infant born at 24 weeks gestation who eventually succumbs to the complications of prematurity after a lengthy course of neonatal intensive care.

Therefore, a comprehensive standardised protocol on investigation of neonatal deaths accommodating all scenarios is not feasible or appropriate. The decisions regarding appropriate investigations should be made by the clinical team providing care based on the individual circumstances accessing additional specialist expertise as required, such as a Neonatologist (if the death occurs outside a tertiary centre), clinical geneticist and metabolic physician.

However, the importance of a high quality autopsy in accurately determining the cause of a neonatal death must be stressed. Neonatal care providers are encouraged to discuss the value of an autopsy with the parents for all neonatal deaths.

(For further discussion on post-mortem examination and placental pathology please refer to Section 4 Perinatal post-mortem examination.)

For neonatal deaths which are considered to be near miss stillbirths, the maternal investigations related to a felt death in-utero should be considered.

Investigation to identify the cause of neonatal death should ideally commence at the birth of all high risk infants. In this section of the guideline, a list of core investigations is provided, based on a consensus of the Working Party, which should be undertaken at the birth of high risk newborns. Investigations which may be considered in certain clinical scenarios are also provided. Investigations at the time of birth are particularly important for infants who survive for only a short time and where an autopsy examination is not undertaken.

High risk newborns include the following:

- Admissions to neonatal intensive care;
- Preterm birth less than 32 weeks gestation;
- Suspected fetal compromise including growth restriction;
- Severe cardiorespiratory depression at birth;
- Signs consistent with congenital infection;
- Severe growth restriction;
- Hydropic infants;
- Suspected severe anaemia;
- Suspected or known major congenital abnormalities; and
- Other circumstances where a liveborn infant dies shortly after birth in the delivery room.

A subgroup of the Guideline Working Party have worked collaboratively in the development of this Section, the members were: Alison Kent, Lucy Cooke, David Tudehope, Ross Haslam, Jane Dahlstrom and Adrienne Gordon.

6.2 Recommended minimal investigations for all neonatal deaths

Clinicians should discuss the value of an autopsy with the parents in all cases of a neonatal death and offer the option of the procedure. The investigation of a neonatal death should include, as a minimum, a thorough maternal and infant medical history

(Please see Section 4: Perinatal postmortem examination.)

A newborn screening blood sample should be performed for all neonatal deaths if not undertaken before the death occurred.

A detailed external examination of the baby should be performed by a perinatal pathologist or an experienced Neonatologist or paediatrician where possible.

(Please see Section 2; Appendix 1.4 Clinical examination of baby checklist)

6.3 Recommended core investigations for high risk newborns

Close collaboration between the obstetric and neonatal care teams is required to ensure that relevant maternal and neonatal factors are considered in the investigation of the neonate.

The following core investigations are recommended at the birth of high risk infants::

- *A detailed external examination of the baby by a Neonatologist or Paediatrician (where possible) with clear documentation of the findings in the medical record;*
- *A comprehensive maternal medical, social and antenatal history including the results of investigations should be documented in the medical record by the obstetric staff;*
- *Cord blood gas analysis including both arterial and venous samples;*
- *Newborn screen*
- *A detailed macroscopic examination of the placenta and cord and documentation of the findings in the medical record by the obstetric staff; and*
- *Placenta, cord and membranes sent fresh and unfixed to pathology for histopathological examination.*

6.4 Further investigations for high risk newborns at the time of birth

Further investigations at the time of birth may provide valuable information in specific situations, particularly in the event of neonatal death, where consent for autopsy is not obtained.

These scenarios and investigations include:

6.4.1 Suspected congenital infection including birth after clinical chorioamnionitis and spontaneous preterm labour and delivery

- Maternal low vaginal/anorectal culture for Group B streptococcus (GBS) and vaginal culture for other common bacterial pathogens associated with perinatal death (e.g. E coli , Klebsiella);
- Maternal blood cultures if clinical chorioamnionitis suspected
- Maternal serology for Cytomegalovirus, Toxoplasma, Parvovirus B19, Rubella and Syphilis (if not undertaken in this pregnancy);
- Infant blood samples for haematological assessment (full blood count with nucleated red cell count), CRP, blood group, DCT and antibody screen and microbiological culture;
- Infant surface swabs from the ear and throat for microbiological cultures;
- Placental swabs between the amnion and chorion using aseptic technique for aerobic and anaerobic bacterial and fungal cultures;
- Placental histological examination specifically for maternal and fetal inflammatory responses and
- If viral infection is suspected a placental biopsy should be sent for appropriate PCR or viral culture.

6.4.2 Suspected congenital abnormalities, hydropic and severely growth restricted infants

- Maternal serology for Cytomegalovirus, Toxoplasma, Parvovirus B19, Rubella and Syphilis (if not undertaken in this pregnancy);
- Infant blood samples for haematological assessment (full blood count with nucleated red cell count), blood group, DCT and antibody screen and microbiological culture and CRP;
- Infant surface swabs from the ear and throat for microbiological cultures;
- Placental swabs between the amnion and chorion using aseptic technique for aerobic and anaerobic bacterial cultures;
- If viral infection is suspected a placental biopsy should be sent for appropriate PCR
- Infant cord or peripheral blood sample for chromosomal analysis;
- Placental histopathology to determine possible causes for growth restriction ⁽¹⁾
- Clinical photographs; and
- For hydropic infants blood test for Transferrin Isoforms for Carbohydrate deficient glycoprotein disorders (CDG)

Following consent from the parents, clinical photographs should be taken for later review, particularly in the circumstance of birth in non tertiary hospital settings. These photos are additional to the bereavement photographs, and should be clearly labelled and filed in the medical record. The use of digital imaging for this purpose is optimal, however issues regarding storage and patient confidentiality should be considered.

6.4.3 Severe cardiorespiratory depression

- Maternal low vaginal/anorectal culture for GBS and vaginal culture for other common bacterial pathogens associated with perinatal death (e.g. E-coli, Klebsiella)⁽²⁾;
- Maternal serology for Cytomegalovirus, Toxoplasma, Parvovirus B19, Rubella and Syphilis if not undertaken in this pregnancy;
- Infant blood samples for haematological assessment (full blood count with nucleated red cell count); blood group, DCT and antibody screen and microbiological culture;
- Infant surface swabs from the ear and throat for microbiological cultures;
- Placental swabs between the amnion and chorion using aseptic technique for aerobic and anaerobic bacterial and fungal cultures. (See Section 2 Institutional Perinatal Mortality Audit, Appendix 1 Perinatal Mortality Audit Package for instructions on taking a placental culture); and
- Consider investigation for genetic metabolic disorder and blood sample for chromosomal analysis.

6.4.4 Suspected thrombophilic disorders: pre-eclampsia, fetal growth restriction

Studies to identify possible thrombophilic disorders should be considered in mothers with preeclampsia or with a personal/family history of thrombosis, or following the birth of an infant with severe growth restriction^(1, 2). These should include initial testing followed by further testing at 8-12 weeks postpartum as required. Selective screening for thrombophilic disorders following birth of high risk neonate or a neonatal death may be helpful in assisting parents and clinicians in understanding the cause of death, planning future pregnancies and giving consideration to the risks and benefits of antithrombotic therapy⁽³⁾.

Screening:

- At birth: Anticardiolipin antibodies; Lupus anticoagulant; Activated protein C (APC) resistance. These tests are recommended at birth as antiphospholipid antibodies may become negative one to two months after pregnancy, and more importantly a number of women may not return for follow-up;
- 8 to 12 weeks postpartum: If antiphospholipid antibodies were present at birth, the test should be repeated, also a fasting Homocysteine and test for Protein C and S deficiency and Prothrombin mutation G20210A and Anti-thrombin III should be undertaken;
- If the APC resistance is positive testing for factor V Leiden gene mutation should be undertaken;
- If the homocysteine test is positive testing for Methylenetetrahydrofolate reductase (MTHFR) should be undertaken.
- MTHFR3 mutation testing should also be performed when the following fetal anomalies are identified - cleft lip/palate, neural tube defects and cardiac defects.

*NB the recommended testing for 8 to 12 weeks postpartum may be performed at birth where the above specific conditions eg fetal growth restriction are known.

(See Section 4 Investigation of a stillbirth for further discussion on Thrombophilia.)

6.4.5 Macrosomic infant

Investigation for maternal diabetes, if not previously undertaken, should include:

- Maternal HbA_{1c} level (as soon as possible after delivery); and
- If the HbA_{1c} level is raised, a fasting blood glucose should be undertaken and if abnormal a Glucose Tolerance Test performed 6-8 weeks postnatally.

The increased risk of perinatal morbidity and mortality with maternal diabetes is well known. As universal screening for diabetes is not currently implemented throughout Australia and New Zealand it is essential that the possibility of undiagnosed maternal diabetes is excluded. HbA_{1c} monitors glycaemia over the previous 3 months by reflecting the average glucose concentration over the life of the red cells.

If there is no clear maternal diabetic history, other causes of macrosomia such as Beckwith Wiedemann syndrome with close look for syndromic features and placental changes should be considered.

6.4.6 Suspected genetic metabolic disorders

To ensure a precise diagnosis, peri mortem evaluation of infants suspected of having genetic metabolic disorders is required. Parental consent is required for a post-mortem examination and for tissue and blood samples to be taken prior to the death. Clinicians need to counsel parents sensitively about the importance of an accurate diagnosis for future genetic risks in this very distressing time.

Due to the complexity and number of different possible diseases, it is strongly recommended that clinicians discuss each individual case with the State Laboratory to identify the optimum tests to request. Should more expert guidance be required a clinical metabolic specialist should be consulted.

All tissue samples should be stored and transported to a Specialist Metabolic Laboratory for investigation as convenient.

Peri-mortem investigation by the clinician should include the following:

Prior to death (Section 6; Appendix 2a Screening for genetic metabolic disorders)

- Seek consent from the parents for a metabolic autopsy;
- Consult metabolic physician or histopathologist before collection of samples;
- Blood sample (0.8ml) in a lithium heparin tube and refrigerate;
- Urine sample (5-10 ml); and
- Knee cartilage and or Skin biopsy (3 x 2 mm punch biopsies): and sent to cytogenetics with request for fibroblast culture and store.
- Liver and muscle biopsies (for electron microscopy, histopathology and enzymology)

Christodoulou and Wilcken⁽⁴⁾ highlight the need for an increased index of suspicion for genetic metabolic disorders (inborn errors of metabolism) in neonatal cases. The authors describe predominant clinical or biochemical presentations of genetic metabolic disorders in the neonatal period and recommend a protocol for screening for these disorders and also for a genetic autopsy.

(Please see Section 6; Appendix 2b Components of the genetic autopsy for details of a genetic autopsy).

The predominant clinical or biochemical presentations of genetic metabolic disorders are as follows: acute encephalopathy; hypoglycaemia, hyperammonaemia, ketosis, disorders of acid-base balance, seizures as an early predominant feature; acute hepatocellular disease; sudden death; severe hypotonia; non-immune hydrops fetalis; facial dysmorphism, with or without congenital malformations⁽⁴⁾.

(Please see Section 6; Appendix 1 High risk newborn investigation checklist for the investigations checklist; Section 2 Institutional Perinatal Mortality Audit, Appendix 1 Perinatal Mortality Audit Package 1.3 for instructions on placental examination and culture technique and Section 2 Institutional Perinatal Mortality Audit, Appendix 2 Instructions for taking clinical photographs for instructions on taking clinical photographs.)

6.4.7 Sudden unexpected neonatal death

The investigation of a sudden unexpected neonatal death should include, as a minimum, a thorough maternal and infant medical history; and a full autopsy examination by a forensic pathologist skilled in perinatal autopsy or a forensic pathologist in conjunction with a perinatal pathologist. An investigation of the various scenes where incidents leading to the death might have occurred including the infants sleeping environment.

For all sudden unexpected neonatal deaths, investigation for genetic metabolic disorders should be undertaken.

The sudden unexpected death of a neonate requires comprehensive investigation as to the cause of the death. Although, Sudden Infant Death Syndrome (SIDS) is rare in the neonatal period since implementation of Back to Sleep campaigns, there has been a proportionate increase in the number of cases occurring at less than one month of age^(4, 5). It is important that all unexpected deaths are investigated fully prior to designation to the category of SIDS and the role of infection/inflammation examined⁽⁹⁾. Based on epidemiological and population characteristics classification of these deaths is incorporated into the PSANZ classification systems^(7, 8). The classification includes a category for unclassified sudden death where no cause for the death was identified and where inadequate

investigation was undertaken. Classification of deaths into this category will hopefully decrease in number with appropriate investigations ensuring that a diagnosis is found in most cases.

The Royal College of Pathologists and The Royal College of Paediatrics and Child Health have published a comprehensive protocol for care and investigation for sudden unexpected deaths in infancy. Please refer to this document for further details⁽⁶⁾.

(For further details on the classification of SIDS, please refer to Section 7 Perinatal death classifications.)

6.5 Alternative investigations where permission for autopsy is not obtained

If permission for an autopsy is not obtained, other less invasive testing may assist in establishing whether any important abnormalities have been missed. These alternatives permit detailed investigation of the baby while still respecting the wishes of the parents⁽⁷⁾. However, a Working Group of the Royal College of Paediatrics and Child Health found little evidence for valid alternatives to the paediatric post-mortem⁽⁸⁾. Parents should be informed at the time of consent about the possibility of missing an important finding when a full post mortem investigation is not undertaken.

6.5.1 External examination by a perinatal/paediatric pathologist, clinical geneticists or paediatrician

An examination by an experienced clinician is of particular importance where an autopsy examination is declined⁽⁹⁾. Clinicians should discuss the importance of this examination with the parents and arrange for an appropriately skilled clinician to undertake the examination.

6.5.2 Babygram

Parents who decline an autopsy should be asked for consent to undertake a full body X-ray (Babygram). A Babygram may detect abnormalities (mainly skeletal) which may not be detected on an external examination. The Wisconsin Stillbirth Service Program has estimated that approximately 20% of unselected stillborns will have abnormalities which are detectable on X-Ray⁽⁹⁾.

6.5.3 Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (if available) may be offered to parents who decline an autopsy investigation. The investigation should be undertaken as soon as possible after a stillbirth. Clinicians should explain to the parents that a full autopsy remains the gold standard as the MRI does not supply tissue samples and therefore important information may be missed.

A comprehensive overview presented the advantages and disadvantages of the post-mortem MRI⁽¹⁰⁾. The major advantages of post-mortem MRI included the non-invasive nature of the examination and the detection of pathologies and malformations of the central nervous system. The disadvantages included the lack of tissue sampling; limitations in detection of complex cardiac malformations, and other abnormalities (e.g. tracheo-oesophageal fistula, bowel perforations) which are undetectable by post-mortem MRI; and lack of experience in perinatal post-mortem MRI. The authors concluded that a full autopsy remains the gold standard; however, MRI may play an important role when an autopsy is declined⁽¹⁵⁻¹⁷⁾.

6.5.4 Clinical photographs

Following consent from the parents, clinical photographs should be taken for later review, particularly in the circumstance of birth in non tertiary hospital settings. These photos are additional to the bereavement photographs, and should be clearly labelled and filed in the medical record. The use of digital imaging for this purpose is optimal, however issues regarding storage and patient confidentiality should be considered.

6.5.5 Other alternatives to a full post-mortem

Post-mortem needle biopsy; laparoscopic autopsy and small incision access are other alternatives to a full post-mortem for focussed investigation of suspected abnormalities.

6.6 References

1. McLintock C, North R, Dekker G. Inherited thrombophilias: Implications for pregnancy associated venous thromboembolism and obstetric complications. *Curr Probl Obstet Gynecol Fertil.* 2001 July/August 2001:115-49.
2. McLintock C. Inherited thrombophilias: Implications for pregnancy associated venous thromboembolism and obstetric complications (Updated). Personal communication. 2004.
3. Kujovich JL. Thrombophilia and pregnancy complications. *Am J Obstet Gynecol.* 2004 Aug;191(2):412-24.
4. Christodoulou J, Wilcken B. Perimortem laboratory investigation of genetic metabolic disorders. *Semin Neonatol.* 2004 Aug;9(4):275-80.
5. Krous HF, Beckwith JB, Byard RW, Rognum TO, Bajanowski T, Corey T, et al. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. *Pediatrics.* 2004 Jul;114(1):234-8.
6. The Royal College of Pathologists and The Royal College of Paediatrics and Child Health. Sudden unexpected death in infancy: A multi-agency protocol for care and investigation. London: The Royal College of Pathologists and The Royal College of Paediatrics and Child Health 2004 September.
7. Raffles A, Ropel C. Perinatal and infant postmortem examination. Non-invasive investigations are also helpful if permission for a necropsy is refused. *BMJ.* 1995 Apr 1;310(6983):870.
8. Royal College of Paediatrics and Child Health. The future of paediatric pathology services: fetal, perinatal and paediatric pathology; a critical future. Report of a working group to restore and develop specialist paediatric pathology: a critically important specialty, essential for the best quality care of children. London: Royal College of Paediatrics and Child Health 2002 March.
9. Wisconsin Stillbirth Service Program UoW. Guide to etiologic evaluation of the stillborn infant: The WiSSP Protocol. Wisconsin: Wisconsin Stillbirth Service Program; [cited 2004]; Available from: <http://www.wisc.edu/wissp/guidetoe.htm>.
10. Huisman TA. Magnetic resonance imaging: an alternative to autopsy in neonatal death? *Semin Neonatol.* 2004 Aug;9(4):347-53.

Investigations at birth		Risk criteria at birth								
		Preterm	Suspected Infection	Suspected Congenital Abnormalities	Hydropic Infant	Cardio-respiratory depression	Pre-eclampsia Hypertension	Fetal growth restriction	Macrosomia	Suspected Metabolic Disorder
Core	Maternal History	+	+	+	+	+	+	+	+	+
	External exam of neonate	+	+	+	+	+	+	+	+	+
	Macroscopic and microscopic examination. of placenta and cord	+	+	+	+	+	+	+	+	+
	Cord Gas	+	+	+	+	+	+	+	+	+
Baby	Placental Culture	+	+	+	+/-	+		+		
	Placental Cytogenetics			+/-		+/-		+/-		+
	Ear, Throat Swabs	+	+	+	+/-	+		+/-		
	FBC	+	+	+	+	+	+	+		+
	CRP		+			+				
	Group DCT	+	+/-	+/-	+	+/-	+/-	+/-		+
	Blood Culture	+	+	+/-	+	+		+/-		
	Clinical photos	+/-	+	+	+	+/-	+/-	+/-	+/-	+/-
	Babygram	+/-		+/-	+/-	+/-		+/-	+/-	+/-
	Blood Chromosomes			+	+	+/-		+/-		
	Genetic Autopsy			+/-	+/-	+/-		+/-		+
Urine									+/-	
Mother	Vag Culture	+	+		+	+		+		
	Torch Serology	+	+	+	+	+		+		
	Thrombophilic Screen				+/-		+	+		
	HbA1c			+/-	+				+	

Section 6; Appendix 2a Screening for genetic metabolic disorders

Extract from: Christodoulou J, Wilcken B. Perimortem laboratory investigation of genetic metabolic disorders. Seminars in Neonatology 2004;9(4):275-280. (4)

Screening investigations that should be performed in an acutely ill neonate suspected of having a genetic metabolic disorder

Urine

- Odour
- Dipstick tests for ketones, pH, sulphite (a)
- Reducing substances (testing for both glucose and non-glucose reducing substances)
- Amino, organic acid screens (including acylglycines)

Blood

- Full blood count/film
- Urea, electrolytes, anion gap, creatinine
- Glucose
- Calcium
- Blood gases
- Liver enzymes
- Uric acid
- Ammonium
- Lactate and pyruvate
- Amino acids (b)
- Carnitine and acylcarnitines (b)

Cerebrospinal Fluid

- Lactate and pyruvate
- Glucose
- Amino acids (b)

In the case of hypoglycaemia collect blood for the following when the child is hypoglycaemic

- Growth hormone
- Cortisol
- Insulin
- Free fatty acids
- β – Hydroxybutyrate
- Acylcarnitine profile
- Urine should always be collected at the time of hypoglycaemia

- (a) Sulphite is very labile. A negative test result does not exclude sulphite oxidase deficiency or the molybdenum cofactor defect.
- (b) These tests should only be ordered after consultation with a biomedical geneticist or metabolic physician.

Section 6; Appendix 2b Components of the genetic autopsy for investigation of metabolic disorders

Extract from: Christodoulou J, Wilcken B. Perimortem laboratory investigation of genetic metabolic disorders. Seminars in Neonatology 2004;9(4):275-280. (4)

Components of the Genetic Autopsy

- Careful family history, including three generation pedigree
- Invite a clinical geneticist with expertise in dysmorphic syndromes to inspect the infant
- Clinical photographs
- Full skeletal survey
- Parental investigations for a haemoglobinopathy
- Maternal investigations for a thrombophilic disorder

Samples to collect from the baby

Blood

- Dried blood spots on filter paper (newborn screening cards, at least two to three cards stored at room temperature but NOT in a plastic bag (for acylcarnitine profile analysis and is a source of DNA))
- Whole blood (5ml in lithium heparin tube (for carnitine, quantitative amino acids, very long chain fatty acids; separated within 20 mins of collection and stored at -70 °C); AND 5ml in EDTA tube (for DNA extraction; can be stored at 4 °C for 48 h) AND 5ml in lithium heparin tube (for chromosome analysis; must be commenced within 4 h of sample collection))

Urine

- Freeze and store (5ml or more if possible, stored at -70 °C; (for amino acid and organic acid profiles, acylglycines, orotic acid))

Cerebrospinal Fluid

- Freeze and store (1ml stored at -70 °C (for amino acid profile))

Skin

- Biopsy: 3x2mm full thickness collected under sterile conditions (DO NOT use iodine-containing preparations) into culture or viral transport, or saline soaked gauze. Store at 4 °C. Best collected within 12 h of death. Cartilage may be taken for culture if there has been a prolonged period after death before biopsies can be taken. Send as soon as possible to a cytogenetics laboratory for fibroblast culture and storage. To be cultured for archiving in liquid nitrogen.

Other biopsies

- Liver and muscle biopsies (for electron microscopy, histopathology and enzymology (for the latter wrap in aluminium foil, snap freeze and store at -70 °C). Collect within 4 h (preferably 2 h) of death. Consult metabolic physician or histopathologist before collection of samples)
- Other tissue biopsies if specific diagnoses are under consideration.