Clinical Practice Guideline
for Care Around
Stillbirth and Neonatal Death

Version 3.1, March 2018

Endorsed by
SECTION 5
INVESTIGATIONS FOR STILLBIRTH

5.1 Introduction

This section of the PSANZ Clinical Practice Guideline for Care Around Stillbirth and Neonatal Death presents recommended investigations to be undertaken following fetal death/stillbirth. Accurate identification of the cause of stillbirth is the cornerstone to prevention and is critically important to parents to help them to understand why their baby has died and to plan future pregnancies. The high proportion of unexplained stillbirths reported globally is an impediment to these goals\(^1\). Wide variation in reported causes of stillbirth internationally has been attributed to different approaches to investigation. However, the classification system used also plays a role\(^2\). Please refer to Section 7 of the PSANZ Clinical Practice Guideline for Care Around Stillbirth and Neonatal Death for further details on classification of stillbirths and neonatal deaths.

This update of the guideline has been undertaken through a partnership between PSANZ and the NHMRC Stillbirth Centre of Research Excellence in Stillbirth.

In this section of the PSANZ Guideline for Care Around Stillbirth and Neonatal Death, for practice recommendations to assist clinicians to implement the guideline recommendations see Appendices A - T.

5.2 Objective of this section

The objective of this section is to provide guidance on the investigations to perform following a stillbirth to identify an accurate cause of death. Detailed objectives are:

- To provide quality information for parents to help understand why the death occurred;
- To inform future pregnancy planning;
- To inform care in future pregnancies; and
- To inform strategies for prevention at the population-level, and future research.

5.3 What has changed in this update?

The findings of a recent literature review have informed changes in this update. Readability has also been enhanced and reduced duplication across the different sections of the guidelines.

Several investigations previously recommended as core investigations following all stillbirths are now recommended as selective investigations only. These investigations (e.g. thrombophilia studies, tests for infectious diseases, Haemoglobin A1c (HbA1c), liver function and bile acid tests) should be undertaken on the basis of the results of core investigations. Maternal ultrasound scan, full blood count, renal and thyroid function testing are no longer recommended investigations for determining the causes of stillbirth. Amniocentesis for cytogenetic analyses and microbiological studies are no longer recommended.

5.4 Research gaps

Due to the lack of high-quality studies on appropriate diagnostic testing following stillbirth, contentious issues remain and, consequently, components of stillbirth investigation protocols internationally vary\(^3,4\). While there are limited data on costs of stillbirth investigations, one study from the US by Michalski et al in 2002 suggest that a specific comprehensive investigation

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1. Michalski et al, 2002

2. Stillbirth Centre of Research Excellence in Stillbirth


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protocol was of sufficient economic value to be incorporated into routine antenatal care. Others report that a selective or sequential approach to testing may be appropriate. The findings of the National Health and Medical Research Council (NHMRC)-funded study on the yield and costs and benefits of stillbirth investigations across maternity settings in Australia and a Cochrane systematic review of the evidence for test protocols are expected in 2017 and 2018.

5.5 Approach to investigation of stillbirth

The recommended investigations following stillbirth include those that should be routine for the majority of stillbirths (core investigations) and those that should be carried out based on information revealed from core investigations, or in the presence of specific clinical scenarios (sequential or selective investigations) (see Figure 1).

Situations will exist where the cause of a fetal death is already known (e.g. unequivocal diagnosis from prenatal testing), however, as selective investigative approaches may result in important diagnoses being missed, a non-selective approach using the core investigations should be the standard in the absence of unequivocal diagnosis from prenatal testing. The relative merits of not following this approach should be considered on an individual-case basis and involve consultation with the family. Depending on the particular circumstances of a perinatal death (e.g. family wishes, access to services), it may not be feasible for some stillbirth investigations to be carried out.

This Guideline provides checklists and data collection forms to assist clinicians with uniform investigation and reporting of stillbirths.

### Section 5 Recommendations for stillbirth investigations

1. A non-selective approach according to the recommended core investigations should be adopted for all stillbirths (unless the cause of death has been unequivocally determined antenatally). These investigations are:
   - Comprehensive maternal (medical, social, family) and pregnancy history
   - Kleihauer-Betke test/Flow cytometry for fetal to maternal haemorrhage
   - External examination of the baby performed by the attending clinician
   - Clinical photographs of the baby
   - Autopsy
   - Detailed macroscopic examination of the placenta and cord
   - Placental histopathology
   - Cytogenetics (Chromosomal microarray (CMA) or karyotype if CMA is not available).

2. Further sequential and/or selective investigations should be undertaken according to the particular clinical scenario based on a comprehensive history, and information gained from core investigations.

3. An external examination of the baby should be performed at birth by the attending clinician using the recommended checklist (Please refer to Appendix D – Clinical examination of baby checklist) and clearly documented in the medical record. Where the family has consented to autopsy, all information gained from the initial external
examination (along with comprehensive maternal (medical, social, family) and pregnancy history) should be forwarded to the pathology service to guide this procedure.

4 Following a stillbirth, the placenta, membranes and cord should be kept refrigerated and, where feasible, sent fresh and unfixed for macroscopic and histological examination by a perinatal pathologist. The pathology service should be informed if the parents have requested return of the placenta following examination.

5 Clinicians should discuss the value of a full autopsy with parents in all cases of perinatal death where the cause of death is not already known. If the parents decline a full autopsy, a limited/partial autopsy should be offered.
### Core investigations

#### Mother
- Maternal history
- Maternal examination
- Kleihauer-Betke or flow cytometry

#### Baby
- Clinical examination at birth
- Full autopsy

#### Placenta
- Macroscopic examination
- Histopathology studies
- Cytogenetic analysis

### Findings from core investigations

<table>
<thead>
<tr>
<th>Mother</th>
<th>Baby</th>
<th>Placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal or family history of thrombosis</td>
<td>Non-consent for full autopsy</td>
<td>Placental abruption or infarction</td>
</tr>
<tr>
<td>Suspected cholestasis</td>
<td>LGA</td>
<td>Infection</td>
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### Indicated selective investigations

- APS (anticardiolipin, lupus anticoagulant, anti-B2 glycoprotein-1 antibodies)
- Bile acids; LFTs
- MRI; NIA; MIA; Clinical photographs
- HbA1c
- Infectious diseases (e.g. CMV); HbA1c; APS (anticardiolipin, lupus anticoagulant, anti-B2 glycoprotein-1 antibodies)
- Further testing as directed by pathologist

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**APS:** Antiphospholipid syndrome; **CMA:** Chromosomal microarray; **CMV:** Cytomegalovirus; **FGR:** Fetal growth restriction; **LFTs:** Liver Function Tests; **LGA:** Large-for gestational-age; **HbA1c:** Haemoglobin A1c; **MIA:** Minimally-invasive autopsy; **MRI:** Magnetic Resonance Imaging; **NIA:** Non-invasive autopsy; **SGA:** Small for gestational age.

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**Figure 1: Stillbirth investigations algorithm**
5.6 Core investigations for all stillbirths

After the diagnosis of stillbirth, and at an appropriate time, the responsible clinician should explain to the parents the value of performing the recommended core investigations as outlined below.

Maternal history

A comprehensive maternal (medical, social, family) and pregnancy history should be taken following all perinatal deaths.

Please refer to Appendix E – Australian perinatal mortality audit tool; and Appendix G – New Zealand Rapid reporting form for a perinatal death - mother

Fetal-maternal haemorrhage (FMH)

In a large study from Ireland over 25 years, fetal-maternal haemorrhage (FMH) accounted for 4.1% of antepartum stillbirths, mostly at term gestations (74%), with multiple gestations being up to six times as likely to be affected as singleton pregnancies. Another case controlled study also noted that women with FMH were more likely to work outside the home during pregnancy than women without evidence of FMH. By general consensus, >50ml is considered a significant fetal haemorrhage, although various studies use limits ranging from 30 to 150ml. However, as the impact of a haemorrhage of a given volume is dependent on gestational age, fetal size and total blood volume, individual assessments need to be calculated.

A Kleihauer-Betke test or flow cytometry to detect FMH should be performed following the diagnosis of any fetal death, preferably prior to birth. Limited evidence suggests that this may also be useful postpartum.

Please refer to Appendix B – Estimation of severity of feto-maternal haemorrhage.

Autopsy

Please refer to Section 4 Perinatal autopsy examination for further details on the post-mortem examination and Section 3 Psychological and social aspects of perinatal bereavement, Appendix M – Infant autopsy consent brochure and Appendix N – Information for health professionals seeking consent for information brochures for parents and professionals about post-mortem examinations.

The following should accompany the infant for autopsy examination:

- Autopsy consent form
- Placenta (fresh and unfixed)
- Comprehensive maternal (medical, social, family) and pregnancy history
- Copies of the death certificate
- Copies of all antenatal ultrasound reports (including post-mortem ultrasound if undertaken)
- Copy of prenatal karyotyping results if available
- Findings from initial external examination performed at birth by attending clinician using the checklist provided (Please refer to Appendix D – Clinical examination of baby checklist).
External examination of the baby

A comprehensive external examination of the baby is an essential component of the investigation of a stillbirth\textsuperscript{15,20-28}. A report on a large case series from the Wisconsin Stillbirth Service Program (WiSSP) suggested that approximately 25\% of stillborn infants were found, on clinical examination, to have demonstrable abnormalities and also indicated that lack of external examination would have resulted in approximately 4\% of diagnoses being missed\textsuperscript{29}.

A detailed external examination of the baby is a component of a full autopsy. As the perinatal pathologist is the most appropriate person to carry out the external examination, parents who have declined a full autopsy should be asked for their consent for the baby to have a detailed external examination by a perinatal pathologist. This should not replace the initial external examination performed at birth by the attending clinician, which may provide important information to guide the full autopsy. In the circumstance where it is not possible for a pathologist to perform the examination, then a neonatologist or paediatrician or clinical geneticist should conduct the examination. A proforma is provided to assist the midwife/doctor in carrying out the procedure.

*Please refer to Appendix D – Clinical examination of baby checklist.*

Clinical photographs

Clinical photographs should be taken for every stillborn baby for later review. The clinical photographs are additional to the bereavement photographs, and should be clearly labelled and filed in the medical record. The WiSSP case series indicates that 28\% of all stillborn babies had observable abnormalities identifiable on photographs and photographs were critical in establishing a diagnosis in approximately 5\% of cases\textsuperscript{29}. Consent from the parents for clinical photographs should be documented in the medical record.

*Please refer to Appendix H – Instructions on taking clinical photographs*

Placental and cord investigations by clinician

At time of birth, the clinician should undertake:

- A detailed macroscopic examination of the placenta and cord and document the normal and abnormal findings;
- Sampling of cord and placental tissue for chromosomal analysis (only if the placenta is not being sent to the pathology service). If a prenatal karyotype has already been performed, these samples should still be taken for DNA extraction and storage;
- Following a stillbirth, the placenta, membranes and cord should be sent fresh and unfixed for macroscopic and histological examination. If removal of an entangled cord from the baby is necessary, then clinical photographs should be taken first.

*Please refer to Appendix C – Placental examination; Accoucheur flow chart.*

Placenta, membranes and cord histopathology

Examination of the placenta, membranes and cord should occur for all stillbirths. The placenta, membrane and cord should be sent to the pathologist fresh and unfixed for histopathological examination once samples have been collected for cytogenetics and microbiology. A perinatal/paediatric pathologist should undertake the examination\textsuperscript{30}. For further details, please
refer to Section 4. A standardised reporting form for placental histopathology is provided to enable high quality reporting (See Appendix P – Placental histopathology reporting form).

Cytogenetic investigations

Chromosome microarray (CMA), in contrast to conventional culture karyotyping, uses DNA and does not require viable cells, which means that chromosomal abnormalities can be detected in macerated stillbirth samples\(^ {31-33}\). Microarray is also superior to karyotype as it can detect additional genetic abnormalities, including microdeletions and microduplications\(^ {32}\). Targeted genetic testing using fetal and/or placental DNA (e.g. for monogenic disorders) will always have a place where a specific phenotype is suspected or when the family history is informative.

Advances in molecular genetic testing using DNA have facilitated a greater depth of testing of both the fetal and placental genome. New technologies currently under evaluation include exome and whole genome sequencing\(^ {34}\). Such advances have highlighted the importance of storing fetal and placental DNA for later evaluation where a cause for fetal death remains unknown.

Specific advice around extended genetic investigation cannot be prescribed at this time as data are limited, and access is variable and costs are generally high. It is therefore recommended that each service explore genetic testing options available to them both locally and further afield through transportation of tissue or DNA samples.

5.7 Selective investigations based on findings of core investigations

Congenital infections

Routine testing of all stillbirths for infection is no longer recommended\(^ {7,18}\). Targeted investigation should be undertaken if infection is suspected on the basis of maternal history, autopsy and/or placental findings\(^ {18}\) and/or a small for gestational age (SGA) baby. To assign infection as the cause of death, positive serology should be supported by autopsy and/or placental findings consistent with infection\(^ {17,18}\). Detailed information about specific infections is outlined below.

Cytomegalovirus (CMV)

Cytomegalovirus is the most frequent infectious cause of newborn developmental abnormalities in the developed world\(^ {35}\). A prospective study of more than 10,000 women found an increase in fetal loss associated with infection in early pregnancy\(^ {36}\). CMV DNA can be detected in a high proportion of fetal or placental tissue samples and a strong association between CMV infection in pregnancy and stillbirth is suggested\(^ {37}\). CMV can also infect the placenta and is associated with villitis\(^ {38}\). Congenital CMV may also be discovered in cases where there are no obvious macroscopic sequelae\(^ {39}\). Maternal CMV serology CMV should be considered where placental histopathology shows evidence of CMV infection and/or when the baby is SGA.

Toxoplasmosis

Congenital toxoplasmosis can cause miscarriage, stillbirth, neurological disability and visual impairment but the majority of fetuses infected will not have sequelae. As toxoplasmosis is not
a common cause of stillbirth\textsuperscript{40}, routine testing in the absence of other indications in not recommended.

\textit{Parvovirus (B19)}

Parvovirus (B19) can cause severe fetal anaemia, nonimmune hydrops and fetal death\textsuperscript{40,41}. One study found parvovirus to be the cause of death in 10\% of all non-malformed fetal deaths that occurred between 10 and 24 weeks of gestation and referred for pathological examination\textsuperscript{42}. A small proportion of susceptible pregnant women (1\%-3\%) will develop serologic evidence of parvovirus infection in pregnancy, of which the transmission rate to the fetus is between 17\% and 33\%\textsuperscript{43-45}. The spontaneous loss rate of fetuses affected by Parvovirus B19 after 20 weeks gestation is 2.3\%\textsuperscript{44,46,47}. However, where parvovirus has caused stillbirth, signs of the disease will be evident following examination of the baby and/or placenta. Routine testing following stillbirth in the absence of other indications is therefore not recommended. Testing for parvovirus should be recommended where severe anaemia and/or non-immune hydrops is found\textsuperscript{7,18}.

\textit{Rubella}

Rubella is associated with a wide variety of fetal abnormalities including stillbirth\textsuperscript{48,49}. However with universal vaccination, congenital rubella infection in developed countries is rare\textsuperscript{50}. Most pregnant women are immune and, if they have not been tested during the initial routine antenatal blood testing, testing for rubella should be done only if indicated on the basis of core investigations.

\textit{Syphilis}

Congenital syphilis may result in fetal loss/neonatal death, prematurity and major long term sequelae in surviving children. In a South American study, even after controlling for congenital anomalies, gestational age, maternal age, and previous stillbirth, gestational syphilis was significantly associated with stillbirth (odds ratio 1.88, 95\% confidence interval 1.25-2.83; \( P=0.002 \))\textsuperscript{51}. This increase in mortality with congenital syphilis has also been confirmed in a systematic review on this topic\textsuperscript{52}. Antenatal screening for syphilis for all women is currently recommended to facilitate treatment early in pregnancy. Syphilis remains an uncommon cause of stillbirth in developed settings\textsuperscript{18} and routine testing following stillbirth in the absence of other indications is not currently recommended.

\textit{Other investigations}

\textit{Blood group and antibody screen}

A maternal blood group and antibody screen is recommended as a routine antenatal test at booking and again in the 3\textsuperscript{rd} trimester of pregnancy. If a blood group and antibody screen has not been performed antenatally, it should be performed selectively to exclude haemolytic disease of the fetus due to maternal sensitisation to red cell antigens\textsuperscript{53} where the baby is anaemic, jaundiced and/or hydropic.
**Thrombophilia testing**

Inherited thrombophilia\(^{54}\) has been associated with a number of adverse pregnancy outcomes including stillbirth, but the relationship remains controversial and the role of testing is still debated. The role of treatment for women with inherited thrombophilia and adverse pregnancy outcomes also remains controversial\(^{55-57}\).

The most common acquired thrombophilia is the antiphospholipid syndrome (APS). The diagnosis of APS includes venous or arterial thrombosis and/or fetal loss in the presence of antiphospholipid antibodies (anticardiolipin, lupus anticoagulant, anti-B2 glycoprotein-1). Confirmation of the presence of antiphospholipid antibodies is required at a minimum of 12 weeks apart, after an initial positive test. APS is associated with both early and late fetal loss, pre-eclampsia and placental insufficiency. The prevalence of antiphospholipid antibodies is estimated to be 5% while the syndrome affects 0.5%\(^{58}\). In a systematic review, the presence of antiphospholipid antibodies was significantly associated with late fetal loss\(^{59}\).

The presence of thrombophilia may be most significant where placental pathology is apparent\(^{18}\). In a small prospective cohort study, 64% of women who had a stillbirth of placental cause had at least one thrombophilia\(^{60}\). This proportion was increased to 70% when looking specifically at preterm stillbirths of placental cause. A population based study in Finland found an almost four-fold increased risk of Factor V Leiden mutation among women with unexplained stillbirth\(^{61}\). The prevalence of this mutation was over ten-fold among women with an unexplained stillbirth in the presence of placental lesions. More recently, a population-based case-control study within the Stillbirth Collaborative Research Network found the only heritable thrombophilia associated with stillbirth was Factor V Leiden mutation, and the association was statistically weak\(^{55}\).

Given the evidence, routine testing for thrombophilic disorders following stillbirth without other indications is not currently justified. Testing for APS (anticardiolipin, lupus anticoagulant, and anti-B2 glycoprotein-1 antibodies)\(^{60,62}\) is recommended selectively where stillbirth occurs in the presence of one or more of the following: (1) family history of thrombosis; (2) personal history of venous thrombosis; (3) fetal growth restriction; (4) placental abruption or (5) placental infarction\(^{8,54}\). Other thrombophilia studies, such as Prothrombin G20210A mutation\(^{60,63-65}\) and Factor V Leiden mutation\(^{55,61,65}\), should be carried out as indicated and in accordance with individual jurisdiction guidance.

**Haemoglobin A1c (HbA1c)**

Increased risk of fetal morbidity and mortality for women with pre-existing diabetes is well known. A stillbirth rate of 35 per 1000 births for Type 2 diabetic mothers has been reported\(^{66}\), and a systematic review has shown that women with pre-existing diabetes have an almost three-fold increased risk of stillbirth\(^{67}\). There is some evidence to indicate that gestational diabetes mellitus (GDM) is also associated with increased perinatal mortality\(^{68}\), and poor detection and management of the condition has been documented in association with term antepartum stillbirth\(^{69}\). It is recognised that some women with GDM have unrecognised type 2 diabetes, with unfavourable pregnancy outcomes\(^{70}\). HbA1c provides information about maternal glycaemia over the previous 3 months by reflecting the average glucose concentration over the life of the red cells\(^{71}\). Therefore, it may provide information regarding the contribution of maternal diabetes to a fetal death. One study suggested that pregnant women with an HbA1c of ≥5.4%...
(36 mmol/mol) may have gestational diabetes\textsuperscript{72}. Another study also noted that although HbA1c cannot replace Oral Glucose Tolerance Test (OGTT) in the diagnosis of GDM, it can be used as a screening test if a cut-off of 5.3% is used\textsuperscript{73}. In such women an OGTT should be performed postnatally. Nonetheless, diabetes has not been shown to be a common cause of stillbirth\textsuperscript{7}. Routine HbA1c following stillbirth without other indication is not currently justified\textsuperscript{7,18}. It is recommended that HbA1c be carried out where SGA, FGR or SGA is detected. Please refer to the Australian Diabetes in Pregnancy Society Guidelines or the New Zealand Screening, Diagnosis and Management of GDM Guideline for further information\textsuperscript{68,74,75}.

**Thyroid function test**

Overt hyper and hypothyroidism are both associated with stillbirth\textsuperscript{76}. However the effect on stillbirth of subclinical hypothyroidism or a positive thyroid antibody test in pregnancy is not clear\textsuperscript{77,78}. Due to this very low risk of stillbirth, routine screening of thyroid function in a clinically euthyroid woman after stillbirth is of limited value\textsuperscript{79}.

**Liver function tests and bile acids**

Abnormalities in liver function tests are markers for viral hepatitis, acute fatty liver of pregnancy, HELLP syndrome and obstetric cholestasis (OC)\textsuperscript{80,81}. Obstetric cholestasis (OC) is a pregnancy-specific liver disease, characterised by maternal pruritus and raised serum bile acids. Risk factors for OC include ethnicity, history of previous liver and/or gallbladder disease including hepatitis B and C, prior OC and multiple pregnancy. A large prospective study of has confirmed the association between severe OC and adverse perinatal outcomes including\textsuperscript{82}. The association was maintained despite bile acid testing being non-fasting. Liver function and (non-fasting) bile acid testing is therefore recommended following the diagnosis of fetal death if there is a maternal history of pruritus.

**Drug screen**

Illicit drug use including amphetamine, methamphetamine, cocaine, pethidine, meperidine, hydrocodone, and tetrahydrocannabinolic acid may contribute to a range of adverse pregnancy outcomes, and use of these substances has been associated with a 2-3 fold increased risk of stillbirth\textsuperscript{83}. While screening for illicit substance use is not recommended as a routine investigation following stillbirth, testing should be considered where indicated on the basis of maternal history.

**5.8 Alternative investigations: When permission for full autopsy is not obtained**

Parents should be informed about the possibility of missing an important finding when a full autopsy is not undertaken. If permission for a full autopsy is not obtained, an external examination, X-ray (babygram) and clinical photos (as described above) are important investigations to perform\textsuperscript{6,84}.

Where available, Magnetic Resonance Imaging (MRI) should be offered to parents who decline an autopsy\textsuperscript{85}. MRI may be diagnostic in some cases (e.g. where intracranial abnormalities are detected)\textsuperscript{85-87}. Clinicians should however explain to parents that a full autopsy remains the gold standard, as MRI does not supply tissue samples and therefore important information may be
missed. Other alternatives to a full post-mortem examination including post-mortem needle biopsy; laparoscopic autopsy, and small incision access for focussed investigation of suspected abnormalities.

Please refer to Section 4 Autopsy examination for further details on alternate investigations to autopsy.

5.9 Storage of plasma and amniotic fluid

Unexplained fetal death is currently the subject of extensive research. Storage of placental and fetal DNA, blood and amniotic fluid allows for future testing for other potential factors that are currently not identified. Even if it is not initially possible to provide an explanation as to the cause of death, parents and siblings may benefit from future research findings if material is stored appropriately. Any storage of human samples requires informed consent to be obtained.

The Stillbirth Centre of Research Excellence in Stillbirth is developing a national collaboration in placental sample storage for research purposes.
5.10 References


5.11 Section authors

5.12 Acknowledgements
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