



## Section 5 of 7 - Investigation of Stillbirths

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## SECTION 5 INVESTIGATION OF STILLBIRTHS

### 5.1 Introduction

This section recommends investigations to be undertaken for stillbirths. The investigations are presented according to those which should be considered routine for all stillbirths (Core investigations) and those which may be required depending on results of Core investigations and in particular clinical conditions (Further investigations). The Core investigations are grouped by the timing of the investigation in relation to the death, i.e. at the time of confirmation of an intrauterine fetal death (IUFD) and following the birth. The guideline includes checklists and data collection forms included in the appendices in Section 2 Institutional Perinatal Mortality Audit to assist clinicians in undertaking uniform investigation and reporting of stillbirths.

There will be situations where the cause of fetal death has already been clearly established (e.g. when karyotyping has already been undertaken in the pregnancy) or previous investigation clearly negates the need for certain tests e.g. no requirement to include HbA1C in patients with normal GCT 2 weeks earlier. However, as using a selective investigative approach may result in missed important diagnoses<sup>(1)</sup>, clinicians should use the non-selective approach as the standard (i.e. Core investigations for all stillbirths) and debate the relative merits of not following this approach on an individual case basis. Depending on particular circumstances of the death (e.g. family wishes and access to services) it may not be possible for some investigations to be carried out.

The recommendations have been developed to provide a comprehensive approach to the investigation of stillbirths with the aim of providing better information to assist in discussion with the parents and in the planning of future pregnancies, and to contribute to the body of knowledge in the understanding of factors associated with stillbirth which may help in reducing future pregnancy loss. It is hoped that, with the support of clinicians across Australia and New Zealand (ANZ) with implementation of these recommendations, a high quality comprehensive data set will be available to enhance the value of surveillance and research activities aimed at reducing the risk of stillbirth. Inevitably, given the lack of high quality studies, some contentious issues remain. The Working Party welcomes comments which will assist with further refinement of the recommendations for stillbirth investigation.

A subgroup of the Working Party (Glenn Gardener, Lesley McCowan, James King, Jane Zucculo, Katie Day (nee Waters) Gus Dekker, and Vicki Flenady) drew on existing national and international protocols for stillbirth investigation and the findings of a comprehensive literature search in the development of this section of the guideline.

The main resource documents used in the development of this section were:

1. Queensland Maternal and Perinatal Quality Council. Maternal and perinatal mortality audit: Guidelines for maternity hospitals. Queensland: Queensland Government, Queensland Health; 2003.
2. NSW Health Department. Hospital Procedures for review and reporting of perinatal deaths. Electronic source <http://www.health.nsw.gov.au/fcsd/rmc/cib/circulars/2002/cir2002-6.pdf>; 2002 January.
3. Department of Human Services South Australia. Maternal, Perinatal and Infant Mortality in South Australia 2002. Including South Australian Protocol for investigation of stillbirths. In: Department of Human Services, South Australia; 2002.
4. Wisconsin Stillbirth Service Program UoW. Guide to etiologic evaluation of the stillborn infant: The WiSSP Protocol. In: Wisconsin: Wisconsin Stillbirth Service Program.
5. British Columbia Reproductive Care Program. Perinatal Mortality Guideline 5: Investigation and Assessment of Stillbirths. British Columbia; 1999.

## 5.2 Recommendations and rationale

*A post-mortem examination, including examination of the placenta, by a perinatal/paediatric pathologist should be offered to all parents following stillbirth.*

*Following a stillbirth, the placenta, membranes and cord should be sent to the perinatal pathologist fresh and unfixed for macroscopic and histological examination regardless of whether consent for autopsy has been gained.*

(Please refer to Section 4 Perinatal post-mortem examination for further details, including rationale, on autopsy and placental pathology.)

*A non-selective approach according to a list of recommended Core Investigations should be adopted for all stillbirths. This non-selective approach is defined as investigations which should be undertaken as the standard approach for all stillbirths, debating the relative merits of not following this approach on an individual case basis.*

*Further investigations should be undertaken according to the particular clinical problem (See Item 5.2.2).*

(Please see Section 5; Appendix 1 Stillbirth investigations algorithm.)

### 5.2.1 Core Investigations for all stillbirths

#### (i) **At diagnosis of a fetal death**

- *Comprehensive maternal and family history;*
- *Ultrasound scan to detect possible fetal abnormalities and to assess amniotic fluid volume;*
- *Amniocentesis (where available) for cytogenetic and infection investigation;*
- *Low vaginal and peri-anal swab to culture for anaerobic and aerobic organisms;*
- *Blood tests:*
  - *Full Blood Examination;*
  - *Serology for Cytomegalovirus, Toxoplasma, Parvovirus B19;*
  - *Rubella and Syphilis if not already undertaken in this pregnancy;*
  - *Blood group and antibody screen if not already undertaken in this pregnancy;*
  - *Kleihauer-Betke test;*
  - *Renal Function Tests including Uric Acid;*
  - *Liver Function Tests and Bile Acid;*
  - *Thyroid Function Tests*
  - *HbA1c*
  - *Anticardiolipin antibodies;*
  - *Lupus anticoagulant; and*
  - *Activated protein C (APC) resistance.*

#### **Ultrasound scan**

*At the time of ultrasound confirmation of an IUID, the ultrasound should include examination for possible fetal abnormalities, fetal biometry and assessment of amniotic fluid volume.*

This assessment may be helpful in identifying a cause for the death particularly where an autopsy examination is not performed.

#### **Maternal history**

*A comprehensive maternal medical and social history should be taken following all perinatal deaths.*

*(Please refer to Section 2 Institutional Perinatal Mortality Audit, Appendix 1 Perinatal Mortality Audit Package, 1.5 Perinatal mortality confidential case summary for a maternal history proforma.)*

#### **Amniocentesis for cytogenetic and infection investigation**

Where possible, an amniocentesis should be performed for cytogenetic and infection investigation following diagnosis of an IUID. It is estimated that 6.9%–20% of stillbirths have a fetal chromosomal abnormality, including a wide range of lethal conditions<sup>(1-3)</sup>. Caution should be exercised in utilising a selective approach for cytogenetic assessment as important diagnoses may be missed<sup>(1, 4)</sup>. Tissue samples of the amnion as well as placental villi may maximise the

likelihood of a result if the fetus is macerated (personal communication with Mark Pertile; Head Scientist, Murdoch Institute, Prenatal Diagnosis, Laboratory)<sup>(5)</sup>.

The rate of successful chromosome analysis using amniocentesis in cases of fetal death ranges from 82%-92%<sup>(2, 4)</sup>. In contrast, the success rate for placental chromosome analysis is approximately 60% and approximately 30% for skin<sup>(2)</sup>. The total time elapsed from fetal death until biopsies can be processed is often long, and the chances of succeeding with a chromosomal analysis diminishes progressively with time<sup>(2)</sup>. The Wisconsin Stillbirth Protocol Program (WiSSP)<sup>(6)</sup> study series indicates that the success of karyotyping ranges from 80% in stillbirths without maceration to 30% in stillbirths with mild to advanced maceration<sup>(6)</sup>. Amniocentesis reduces the elapsed time between fetal death and sample collection, and the samples are easier to handle and for the laboratory to process<sup>(7)</sup>.

Amniotic fluid collected by amniocentesis prior to the onset of labour can provide an uncontaminated specimen for microbiological assessment. It is the only sample where the detection of pathogens such as E-coli will be of value, especially if no autopsy is performed. This is due to potential contamination during vaginal birth where findings from cultures of natural orifices and the placenta/membranes are often discredited<sup>(7)</sup>.

***Vaginal cultures: Low vaginal peri-anal culture for anaerobic and aerobic organisms.***

McDonald et al identified that although 70% of women with mid-gestation spontaneous abortions were asymptomatic for infection, micro-organisms were identified from the placenta and/or fetus in 62% of women studied and histological chorioamnionitis was present in 69%. Among 51 women with intact membranes, 28 were culture-positive, with the most frequent isolate being Group B Streptococcus (GBS). In this study, GBS was the most significant pathogen associated with the fetal deaths, and was often the sole pathogen recovered<sup>(8)</sup>. The detection of GBS is optimised with the use of a peri-anal swab in conjunction with a low vaginal swab and the use of specific culture media<sup>(9)</sup>.

Method for GBS culture: Using one single dry swab stick, first take a culture from the introitus and with the same swab stick, take a culture from the anorectal region. Place the swab in Stuarts transport medium and send to laboratory clearly labelled. Swabs may be self collected by the patient<sup>(10)</sup>.

***Full blood examination***

A full blood examination can assist in detection of: infection as a cause of the fetal death<sup>(11)</sup>; maternal anaemia which may indicate conditions such as thalassemia; low platelet levels - a marker for pre-eclampsia; autoimmune diseases such as systemic lupus erythematosus (SLE) and Idiopathic Thrombocytopenia Purpura (ITP)<sup>(12)</sup>; and elevated platelet levels may indicate thrombocytopenia.

***Serology for Cytomegalovirus, Toxoplasma, Parvovirus B19, Rubella and Syphilis***

Serology for Cytomegalovirus, Toxoplasma and Parvovirus B19 should be undertaken following an IUFD. Rubella and Syphilis should also be included if they have not already been undertaken during the antenatal period. Where test results are positive, a microbiologist or infectious disease specialist should be consulted regarding further testing and treatment required.

***Toxoplasmosis***

Maternal-fetal transmission of Toxoplasmosis is dependent on the time of maternal infection. The earlier the fetus acquires the infection the more severe the consequences, however maternal-fetal transmission is more likely to occur later in pregnancy. Disseminated Toxoplasma may cause fetal death<sup>(13)</sup>.

***Parvovirus (B19)***

Parvovirus (B19) causes severe fetal anaemia, nonimmune hydrops and fetal death<sup>(13, 14)</sup>. It was found to be the cause of death in 10% of all non-malformed fetal deaths occurring between 10 and 24 weeks of gestation referred for pathological examination<sup>(15)</sup>. 1%-3% of susceptible pregnant women will develop serologic evidence of infection in pregnancy, of which the transmission rate to the fetus is 17%-33%<sup>(16-18)</sup>. The spontaneous loss rate of fetuses affected by Parvovirus B19 after 20 weeks gestation is 2.3%<sup>(16, 18-20)</sup>.

### **Rubella**

Rubella is associated with a wide variety of fetal abnormalities and also infects the placenta, enhancing the risk of stillbirth<sup>(21, 22)</sup>. However due to widespread vaccination, congenital rubella infection in developed countries is extremely rare<sup>(13)</sup>.

### **Cytomegalovirus (CMV)**

Whether CMV actually causes stillbirth and, if so, the mechanism by which it does so is not clear<sup>(13)</sup>. However, a prospective study of more than 10,000 women found an increase in fetal loss associated with infection in early pregnancy<sup>(23)</sup>.

### **Blood group and antibody screen**

A blood group and antibody screen should be performed to exclude haemolytic disease due to maternal sensitisation to red cell antigens, for example Rh D and Kell<sup>(24)</sup>.

### **Kleihauer-Betke test**

A Kleihauer-Betke test to detect fetomaternal haemorrhage should be performed following the diagnosis of an intrauterine fetal death (IUFD) preferably prior to delivery<sup>(25, 26)</sup>. Limited evidence suggests that post delivery Kleihauer may still be useful<sup>(27)</sup>.

The incidence of massive fetomaternal haemorrhage is <0.1%<sup>(28)</sup>. However the incidence in otherwise unexplained cases of fetal death has been estimated to be as high as 14%<sup>(29)</sup>. The diagnosis of a significant fetomaternal haemorrhage is confirmed by quantification of fetal erythrocytes in maternal blood performed by the Kleihauer test. The general consensus is that 50ml constitutes a significant haemorrhage, with various studies using limits ranging from 30-150ml<sup>(29, 30)</sup>. However, as the impact of a haemorrhage of a given volume will be dependent on the fetal age, weight and total blood volume, individual assessments need to be calculated<sup>(7, 31)</sup>.

The time period over which the haemorrhage occurs will have a direct impact upon the mortality associated with it, according to whether the fetus was able to compensate for the loss in blood volume. However, as it is not currently possible to assess this, a loss of 20% of total fetal blood volume should be considered severe enough to cause fetal mortality<sup>(6)</sup>.

*(Please refer to Section 5; Appendix 2 Estimation of severity of fetomaternal haemorrhage for the calculation of significant haemorrhage.)*

### **Renal Function Tests including Uric Acid**

Elevated uric acid levels early in the third trimester in pre-eclamptic women have been associated with perinatal death and it is therefore recommended to evaluate the contribution of pre-eclampsia to the death. Abnormal renal function is an indicator of possible SLE<sup>(32)</sup> which is associated with a significant increase in fetal morbidity and mortality<sup>(33)</sup>. Uric acid is the most sensitive laboratory indicator of pre-eclampsia<sup>(34)</sup> and is a better predictor of perinatal outcome than blood pressure<sup>(35)</sup>.

### **Liver Function Tests and Bile acid**

Mild liver test abnormalities are a possible marker for obstetric cholestasis. Obstetric cholestasis is associated with a significant increase in the perinatal mortality rate, ranging from 3%-20% as well as a five-fold increased incidence intrapartum fetal distress and pre-term labour<sup>(36, 37)</sup>. Abnormalities in liver function are also a marker for viral hepatitis, cytomegalovirus, and toxoplasmosis. Abnormal liver function has also been associated with acute fatty liver of pregnancy and HELLP syndrome (**H**aemolysis, **E**levated **L**iver function, **L**ow **P**latelets)<sup>(38)</sup>.

### **Thyroid Function Test**

Pregnancy is associated with physiological changes in the thyroid function which may result in thyroid disorders. Thyroid disorders during pregnancy have been associated with adverse health outcomes for both the mother and child, including increased risk of miscarriage, gestational hypertension, low birth weight and fetal death.<sup>(39)</sup>

### **HbA<sub>1c</sub>**

The increased risk of fetal morbidity and mortality with maternal diabetes is well known. A stillbirth rate of 35 per 1000 births to type 2 diabetic mothers has been reported<sup>(40)</sup>. Gestational diabetes mellitus (GdM) is defined as carbohydrate intolerance of variable severity with the onset or first recognition during pregnancy. There is some evidence to indicate that uncontrolled GDM is associated with increased perinatal mortality<sup>(41)</sup>. *HbA<sub>1c</sub>* monitors glycaemia over the previous 3

months by reflecting the average glucose concentration over the life of the red cells<sup>(42)</sup> and therefore may provide information to aid in the consideration of the contribution of diabetes to the fetal death. If the *HbA<sub>1c</sub>* level is raised, a fasting blood glucose should be undertaken and if abnormal a Glucose Tolerance Test performed 6-8 weeks postnatally. Please refer to the Australasian Diabetes in Pregnancy Society GDM management guidelines for further details<sup>(41, 43)</sup>.

#### **Investigation for Thrombophilia**

Anticardiolipin antibodies, Lupus anticoagulant and APC resistance are recommended for all women at the time of IUFD. (Please see Item 5.2.2 (ii) Further Investigation for further details.)

#### **(ii) Following birth**

- External examination of the baby (by a perinatal pathologist, Neonatologist or paediatrician where possible);
- Clinical photographs;
- Surface swabs (ear and throat) for microbiological cultures;
- Post-mortem examination;
- Blood samples from the cord or cardiac puncture for investigation of infection;
- Blood samples for chromosomal analysis;
- Routine Guthrie test
- Detailed macroscopic examination of the placenta and cord;
- Placental microbiological cultures;
- Placental and amnion biopsy for chromosomal analysis; and
- Placental histopathology

#### **External examination of the baby**

A detailed external examination of the baby should be performed by a perinatal pathologist, neonatologist or paediatrician where possible.

A comprehensive external examination of the baby is an essential component of the investigation of a stillbirth<sup>(25, 44-52)</sup>. A report on a large case series from the 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<sup>(6)</sup>.

A detailed external examination of the baby is a component of a full post-mortem. As the perinatal pathologist is the most appropriate person to carry out the external examination, parents who have declined a full post-mortem should be asked to consent for the baby to be examined by a pathologist. In the circumstance where it is not possible for a pathologist to perform the examination, then a neonatologist or paediatrician should conduct the examination. If neither are available, a proforma (Section 2 Institutional Perinatal Mortality Audit, Appendix 1 Perinatal Mortality Audit Package) is provided to assist the midwife/doctor in carrying out the procedure.

#### **Clinical photographs**

Clinical photographs should accompany the detailed external examination of the stillborn.

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 stillborns have observable abnormalities identifiable on photographs and photographs were critical in establishing a diagnosis in approximately 5% of cases<sup>(6)</sup>. Consent from the parents for clinical photographs should be clearly documented in the medical record.

(Please refer to Section 2 Institutional Perinatal Mortality Audit, Appendix 2 Instructions for taking clinical photographs.)

#### **Infant surface swabs for microbiological cultures**

Surface swabs for microbiological cultures should be taken from the ear and throat of all stillbirths.

Intrauterine infections have been reported to represent the cause of death in 15%-24% of cases of stillbirths when premature rupture of membranes are included. Infection may be subclinical in the mother, hence the importance of investigating all incidences of fetal death where a cause is not obvious. Swabs should be taken from the ear and throat of the stillborn and sent for aerobic and anaerobic bacterial culture.

### **Infant blood samples for investigation of infection, chromosomal analysis and Guthrie test screening**

A blood sample should be collected from the infant for investigation of the presence of infection, to assess other haematological parameters for karyotyping (if not already performed) and a routine Guthrie test.

A cord blood sample should be collected after delivery where possible; if this is not possible, cardiac puncture can be performed. This blood sample will provide a potentially uncontaminated sample for microbiological culture and assessment of fetal inflammatory response. If a sample of blood is obtained it should also be sent for chromosomal analysis, and haematological assessment (full blood count, nucleated red cell count, group and antibody screen). If the fetus is macerated samples from the amnion and placenta should also be sent to cytogenetics for chromosomal analysis.

### **Post-mortem examination**

A post-mortem examination by a perinatal pathologist should be recommended to all parents following stillbirth.

The following should accompany the infant for post-mortem examination:

- Post-mortem consent form;
- Placenta;
- Clinical/obstetric history including relevant previous obstetric history;
- Copies of the death certificate;
- Copies of all antenatal ultrasound reports; and
- Copy of prenatal karyotyping results if available

### **Placenta, membrane and cord histopathology**

Following a stillbirth, the placenta, membrane and cord should be sent to the perinatal pathologist fresh and unfixed for macroscopic and histological examination.

(Please refer to Section 4 Perinatal post-mortem examination for further details on the post-mortem examination and Section 3 Psychological and social aspects of perinatal bereavement, Appendices 1 and 2 for information brochures for parents and professionals about post-mortem examinations.)

### **Placental and cord investigations by clinician**

At time of delivery, the clinician should undertake:

- A detailed macroscopic examination of the placenta and cord and document the findings;
- Placental swabs between the amnion and chorion using aseptic technique for aerobic and anaerobic bacterial cultures; and
- Sampling of amnion and placental tissue for karyotyping if required.

If a prenatal karyotype has already been performed, a placental sample for karyotyping is not required.

(Please refer to Section 2 Institutional Perinatal Mortality Audit, Appendix 1 Perinatal Mortality Audit Package for instructions on placental examination and preparation for pathology.)

## **5.2.2 Further investigations for Thrombophilia**

Further investigation for thrombophilia should be undertaken 8-12 weeks postnatally where a fetal death is associated with fetal growth restriction, pre-eclampsia, maternal thrombosis and/or maternal family history of thrombosis, remains unexplained following the core investigations or where tests for thrombophilia were positive at the time of the IUFD as follows:

- Anticardiolipin antibodies; and Lupus anticoagulant repeated if positive at the time of the IUFD or initial testing if not previously undertaken;
- APC resistance if not undertaken at birth;
- Factor V Leiden mutation if APC resistance was positive at birth;
- Fasting Homocysteine and if positive test for \*methylenetetrahydrofolate reductase (MTHFR) gene mutation;

- *Protein C and S deficiency*
- *Prothrombin gene mutation 20210A; and*
- *Anti-thrombin III*

These additional thrombophilia tests may be performed at birth where the above specific conditions eg fetal growth restriction are known. MTHFR mutation testing should be performed when the following fetal anomalies are identified: cleft lip/palate, neural tube defects or congenital cardiac defects<sup>(53)</sup>.

Testing for thrombophilia at 8-12 weeks postnatal should be undertaken where a fetal death is associated with fetal growth restriction, pre-eclampsia, maternal thrombosis and/or maternal family history of thrombosis, if the stillbirth remains unexplained following the core investigations, or thrombophilia tests performed at the time of birth were positive (i.e. Anticardiolipin antibodies, lupus anticoagulant). While the value of screening for inherited thrombophilia remains unclear<sup>(54)</sup>, the Working Party has recommended the following investigations based on the strength of association of many thrombophilic conditions and fetal death<sup>(55)</sup>. It is hoped the implementation of these recommendations for investigation of thrombophilic disorders will not only assist in informing management strategies for future pregnancies, but that this will also enable the establishment of robust prospective data collections across ANZ for research and audit to assist in the understanding of the contribution of thrombophilia to adverse pregnancy outcome and enable monitoring of the effects of interventions to reduce the risk of fetal death.

Thrombophilia is a multigenic disorder caused by inherited and acquired (including a combination of both) defects resulting in a predisposition to thrombosis<sup>(56)</sup>. Antiphospholipid antibodies are the most important causes of acquired thrombophilias. In pregnancy, thrombophilic disorders are associated with an increased risk of venous thromboembolism (VTE), pre-eclampsia, placental abruption, early and late fetal demise, recurrent pregnancy loss and fetal growth restriction<sup>(57, 58)</sup>. Women with inherited combined thrombophilias are at high risk of VTE and poor obstetric outcome as are women with a personal or family history of VTE<sup>(58)</sup>.

Accurate estimates of strength of the associations for adverse pregnancy outcome and inherited thrombophilic disorders are problematic due to small numbers and heterogeneity of the available studies on this broad topic. However, recent systematic reviews have demonstrated a statistically significant increase in the risk of stillbirth associated with: APC resistance<sup>(57, 59)</sup>; Factor V Leiden mutation<sup>(57, 59-61)</sup>; Protein C deficiency<sup>(59)</sup>; Protein S deficiency<sup>(57, 59, 60)</sup>; Prothrombin G20210 mutation<sup>(59, 60)</sup>; and MTHFR<sup>(59)</sup>. One review also demonstrated statistically significant associations with these thrombophilic conditions and pre-eclampsia which was strengthened in the analysis for severe pre-eclampsia<sup>(59)</sup>.

The pathogenesis of unexplained fetal loss in women with thrombophilia is thought to involve uteroplacental insufficiency, thrombosis and infarction. Ideally the identification of thrombophilia following an apparently unexplained stillbirth would result in intervention in future pregnancies to reduce the risk. Although the evidence is unclear, there is emerging data from randomised controlled trials that antithrombotic therapy may reduce adverse pregnancy outcome for women with thrombophilia<sup>(62, 63)</sup>. Therefore, screening for thrombophilic disorders following fetal death may be helpful in assisting parents and clinicians in understanding the cause of the death and in the planning of future pregnancies including consideration of the balance of risks and benefits for antithrombotic therapy<sup>(64)</sup>.

### **5.3 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 fetus or infant while still respecting the wishes of the parents<sup>(65)</sup>. However, a Working Group of the Royal College of Paediatrics and Child Health found little evidence for valid alternatives to the paediatric post-mortem<sup>(66)</sup>. 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.

#### **5.3.1 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<sup>(6)</sup>. Clinicians should discuss the importance of this examination with the parents and arrange for an appropriately skilled clinician to undertake the examination.



### **5.3.2 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<sup>(6)</sup>.

### **5.3.3 Ultrasound scan**

A detailed ultrasound examination of the infant at the time of confirmation of an intrauterine death or after the birth may identify fetal abnormalities which may not be identified by an external examination<sup>(48)</sup>.

### **5.3.4 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 supply tissue samples and therefore important information may be missed.

A recent comprehensive overview presented the advantages and disadvantages of the post-mortem MRI<sup>(67)</sup>. 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.

### **5.3.5 Instrucitons for taking 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.

### **5.3.6 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.

## **5.4 Storage of plasma and amniotic fluid**

Unexplained fetal death is currently the subject of extensive research. Storage of maternal and fetal plasma and amniotic fluid will allow testing for other potential factors in the future, which are currently unidentified, when new discoveries have been made. Therefore, even if it is not possible initially to provide an explanation as to the cause of death, parents and siblings may benefit from research findings in the future. It is essential that informed consent is obtained prior to storage of human samples.

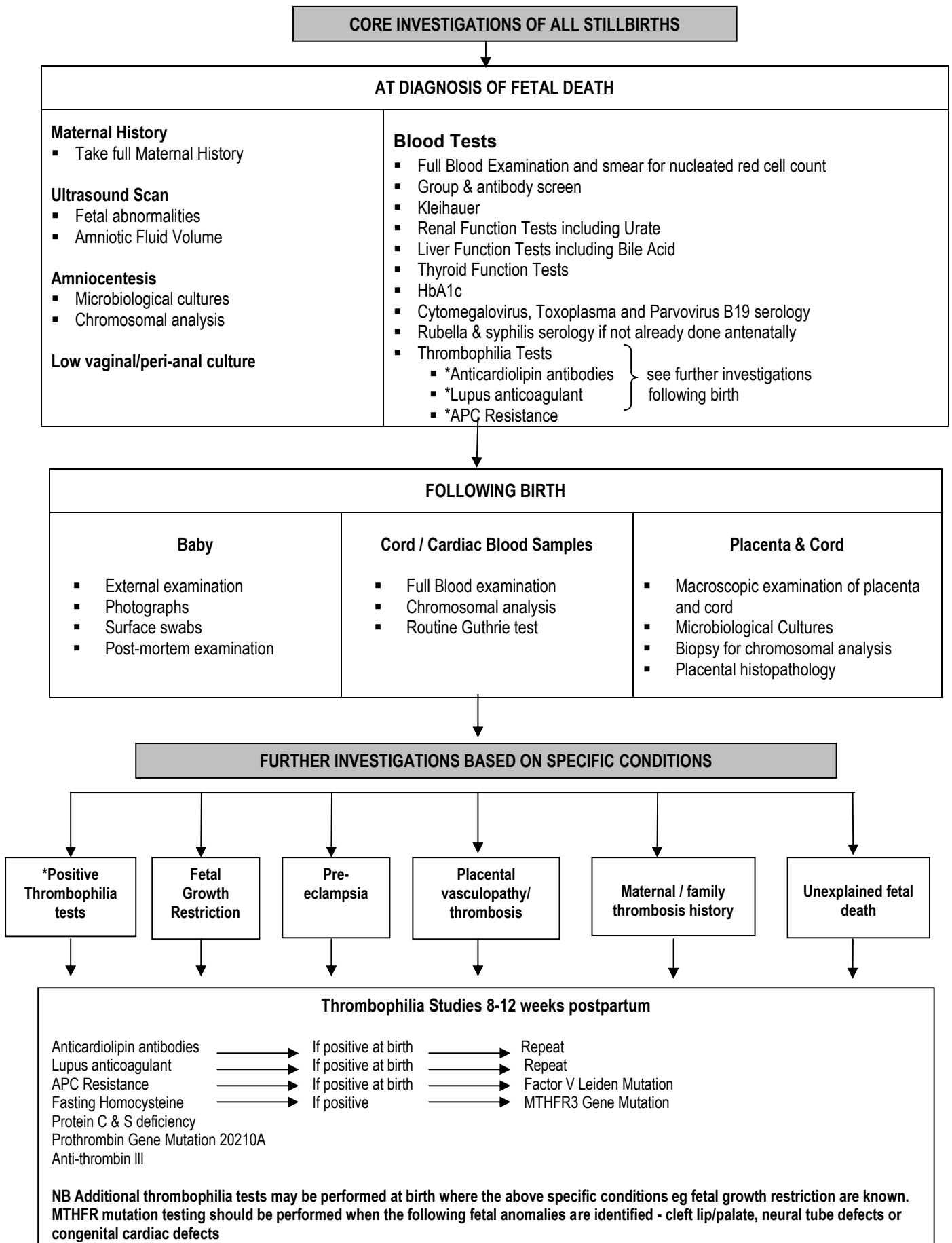
## 5.5 References

1. Smith A, Bannatyne P, Russell P, Ellwood D, den Dulk G. Cytogenetic studies in perinatal death. *Aust N Z J Obstet Gynaecol*. 1990 Aug;30(3):206-10.
2. Ahlenius I, Floberg J, Thomassen P. Sixty-six cases of intrauterine fetal death. A prospective study with an extensive test protocol. *Acta Obstet Gynecol Scand*. 1995;74(2):109-17.
3. Hefler LA, Hersh DR, Moore PJ, Gregg AR. Clinical value of postnatal autopsy and genetics consultation in fetal death. *Am J Med Genet*. 2001 Nov 22;104(2):165-8.
4. Neiger R, Croom CS. Cytogenetic study of amniotic fluid in the evaluation of fetal death. *J Perinatol*. 1990 Mar;10(1):32-4.
5. Pertile M. Samples of amnion are more likely to grow using traditional cytogenetic methods than chorion villi in the macerated fetus. 2005, Personal communication.
6. 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>.
7. Froen JF. Sudden intrauterine unexplained death. Oslo: Unipub AS; 2002.
8. McDonald HM, Chambers HM. Intrauterine infection and spontaneous midgestation abortion: is the spectrum of microorganisms similar to that in preterm labor? *Infect Dis Obstet Gynecol*. 2000;8(5-6):220-7.
9. Jones S, Athan E, Viggers J. Prevention of recurrent fetal death in utero due to group B streptococcal chorioamnionitis. *Aust N Z J Obstet Gynaecol*. 2004 Aug;44(4):356-7.
10. Perinatal Clinical Practice Guidelines Working Party. Clinical Practice Guidelines for the prevention of neonatal early onset group B streptococcal disease. Brisbane: Queensland Health; 2003 [updated 2003; cited]; Available from: [http://qhps.health.qld.gov.au/cpg/guide/Southern\\_Zone/GBS.pdf](http://qhps.health.qld.gov.au/cpg/guide/Southern_Zone/GBS.pdf).
11. Kuvin SF, Brecher G. Differential neutrophil counts in pregnancy. *N Engl J Med*. 1962 Apr 26;266:877-8.
12. Crowther MA, Burrows RF, Ginsberg J, Kelton JG. Thrombocytopenia in pregnancy: diagnosis, pathogenesis and management. *Blood Rev*. 1996 Mar;10(1):8-16.
13. Goldenberg RL, Thompson C. The infectious origins of stillbirth. *Am J Obstet Gynecol*. 2003 Sep;189(3):861-73.
14. Skjoldebrand-Sparre L, Tolfvenstam T, Papadogiannakis N, Wahren B, Broliden K, Nyman M. Parvovirus B19 infection: association with third-trimester intrauterine fetal death. *Br J Obstet Gynaecol*. 2000 Apr;107(4):476-80.
15. Wright C, Hinchliffe SA, Taylor C. Fetal pathology in intrauterine death due to parvovirus B19 infection. *Br J Obstet Gynaecol*. 1996 Feb;103(2):133-6.
16. PHLS Working Party on Fifth Disease. Prospective study of human parvovirus (B19) infection in pregnancy. Public Health Laboratory Service Working Party on Fifth Disease. *BMJ*. 1990 May 5;300(6733):1166-70.
17. Harger JH, Adler SP, Koch WC, Harger GF. Prospective evaluation of 618 pregnant women exposed to parvovirus B19: risks and symptoms. *Obstet Gynecol*. 1998 Mar;91(3):413-20.
18. Crane J. Parvovirus B19 infection in pregnancy. *J Obstet Gynaecol Can*. 2002 Sep;24(9):727-43; quiz 44-6.
19. Rodis JF, Quinn DL, Gary GW, Jr., Anderson LJ, Rosengren S, Cartter ML, et al. Management and outcomes of pregnancies complicated by human B19 parvovirus infection: a prospective study. *Am J Obstet Gynecol*. 1990 Oct;163(4 Pt 1):1168-71.
20. Miller E, Fairley CK, Cohen BJ, Seng C. Immediate and long term outcome of human parvovirus B19 infection in pregnancy. *Br J Obstet Gynaecol*. 1998 Feb;105(2):174-8.
21. Beckwith JB. The value of the pediatric postmortem examination. *Pediatr Clin North Am*. 1989 Feb;36(1):29-36.
22. Benirschke K, Robb JA. Infectious causes of fetal death. *Clin Obstet Gynecol*. 1987 Jun;30(2):284-94.
23. Griffiths PD, Baboonian C. A prospective study of primary cytomegalovirus infection during pregnancy: final report. *Br J Obstet Gynaecol*. 1984 Apr;91(4):307-15.
24. Moise K. Hemolytic disease of the fetus and newborn. In: Creasy RK, Resnik R, editors. *Maternal-Fetal Medicine: Principles and Practice*. 5th Edition ed. Philadelphia: Saunders; 2004. p. 537-61.
25. NSW Health Department. Guidelines for investigation of a stillbirth. Electronic/circular. Sydney: NSW Health Department; 1997 27/10/1997. Report No.: 97/107 Contract No.: Document Number].
26. Nursing and Midwifery Sub-group SZCSN. Antepartum haemorrhage: Southern Zone Maternity Procedural Guidelines. 2003 [updated 2003; cited 2004]; Available from: [http://qhps.health.qld.gov.au/cpg/guide/Southern\\_Zone/APH.doc](http://qhps.health.qld.gov.au/cpg/guide/Southern_Zone/APH.doc).
27. Emery CL, Morway LF, Chung-Park M, Wyatt-Ashmead J, Sawady J, Beddow TD. The Kleihauer-Betke test. Clinical utility, indication, and correlation in patients with placental abruption and cocaine use. *Arch Pathol Lab Med*. 1995 Nov;119(11):1032-7.

28. Van de Putte I, Renaer M, Vermeylen C. Counting fetal erythrocytes as a diagnostic aid in perinatal death and morbidity. *Am J Obstet Gynecol.* 1972 Dec 1;114(7):850-6.
29. Laube DW, Schauburger CW. Fetomaternal bleeding as a cause for "unexplained" fetal death. *Obstet Gynecol.* 1982;60(5):649-51.
30. de Almeida V, Bowman JM. Massive fetomaternal hemorrhage: Manitoba experience. *Obstet Gynecol.* 1994 Mar;83(3):323-8.
31. Pauli RM. Fetal-Maternal Hemorrhage and Stillbirth. [Website] Wisconsin; 1993 [updated 1993; cited 2004 17/05/2004]; Available from: [www.wisc.edu/wissp/wisspers/93940001.htm](http://www.wisc.edu/wissp/wisspers/93940001.htm).
32. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997 Sep;40(9):1725.
33. Yasmeen S, Wilkins EE, Field NT, Sheikh RA, Gilbert WM. Pregnancy outcomes in women with systemic lupus erythematosus. *J Matern Fetal Med.* 2001 Apr;10(2):91-6.
34. Roberts J. Pregnancy-related hypertension. In: Creasy RK, Resnik R, editors. *Maternal-Fetal Medicine: Principles and Practice.* 5th Edition ed. Philadelphia: Saunders; 2004. p. 859.
35. Redman CW, Beilin LJ, Bonnar J, Wilkinson RH. Plasma-urate measurements in predicting fetal death in hypertensive pregnancy. *Lancet.* 1976 Jun 26;1(7974):1370-3.
36. Fisk NM, Storey GN. Fetal outcome in obstetric cholestasis. *Br J Obstet Gynaecol.* 1988 Nov;95(11):1137-43.
37. Rioseco AJ, Ivankovic MB, Manzur A, Hamed F, Kato SR, Parer JT, et al. Intrahepatic cholestasis of pregnancy: a retrospective case-control study of perinatal outcome. *Am J Obstet Gynecol.* 1994 Mar;170(3):890-5.
38. McDonald JA. Cholestasis of pregnancy. *J Gastroenterol Hepatol.* 1999 Jun;14(6):515-8.
39. Feki M, Omar S, Menif O, Tanfous NB, Slimane H, Zouari F, et al. Thyroid disorders in pregnancy: frequency and association with selected diseases and obstetrical complications in Tunisian women. *Clin Biochem.* 2008 Aug;41(12):927-31.
40. Cundy T, Gamble G, Townend K, Henley PG, MacPherson P, Roberts AB. Perinatal mortality in Type 2 diabetes mellitus. *Diabet Med.* 2000 Jan;17(1):33-9.
41. Hoffman L, Nolan C, Wilson JD, Oats JJ, Simmons D. Gestational diabetes mellitus--management guidelines. The Australasian Diabetes in Pregnancy Society. *Med J Aust.* 1998 Jul 20;169(2):93-7.
42. Parfitt VJ, Clark JD, Turner GM, Hartog M. Use of fructosamine and glycated haemoglobin to verify self blood glucose monitoring data in diabetic pregnancy. *Diabet Med.* 1993 Mar;10(2):162-6.
43. Oats JJ, McIntyre HD. Revision of guidelines for the management of gestational diabetes mellitus. *Med J Aust.* 2004 Sep 20;181(6):342.
44. Department of Neonatal Medicine Royal Prince Alfred Hospital. Stillbirths. [Electronic]: Central Sydney Area Health Service; 2004 [updated 2004; cited 2004 2/02/04]; Available from: <http://www.cs.nsw.gov.au/rpa/neonatal/default1.htm>.
45. Manitoba CoPaSo. Investigation of Stillbirths. [Electronic] Manitoba; 1998 [updated 1998; cited 2004 16/02/04]; Available from: [file:///L:%5Cap\\_epi%5CCCS%20STUDIES%5CPerinatal%20Mortality%20Guidelines%5CFetal%20Death%20Invest%20Ch\\_8Oct04.doc](file:///L:%5Cap_epi%5CCCS%20STUDIES%5CPerinatal%20Mortality%20Guidelines%5CFetal%20Death%20Invest%20Ch_8Oct04.doc).
46. Carey JC. Diagnostic evaluation of the stillborn infant. *Clin Obstet Gynecol.* 1987 Jun;30(2):342-51.
47. Department of Human Services South Australia. Maternal, Perinatal and Infant Mortality in South Australia 2002. Including South Australian Protocol for investigation of stillbirths. [Electronic]: Department of Human Services, South Australia; 2002 [updated 2002; cited 2004 2/2/04]; Available from: <http://www.dhs.sa.gov.au/pehs/PDF-files/mortality-report02.pdf>.
48. British Columbia Reproductive Care Program. Perinatal Mortality Guideline 5: Investigation and Assessment of Stillbirths. British Columbia; 1999 Contract No.: Document Number].
49. Alberta MA. Investigation of Stillborn Protocol. [electronic]: Alberta Medical Association; 1998 [updated 1998 25/11/03; cited 2004 2/2/04]; Available from: <http://www.albertadoctors.org/bcm/ama/ama-website.nsf/AllDoc/FB1F65D913EDB64787256E2A005E700E?OpenDocument>.
50. Curry CJ, Honore LH. A protocol for the investigation of pregnancy loss. *Clin Perinatol.* 1990 Sep;17(3):723-42.
51. Kean L. Intra-uterine fetal death. *Curr Obstet Gynaecol.* 2003 2003(13):329-35.
52. Queensland Maternal and Perinatal Quality Council. Maternal and perinatal mortality audit: Guidelines for maternity hospitals. Queensland: Queensland Government, Queensland Health; 2003 Contract No.: Document Number].
53. Kirke PN, Mills JL, Molloy AM, Brody LC, O'Leary VB, Daly L, et al. Impact of the MTHFR C677T polymorphism on risk of neural tube defects: case-control study. *BMJ.* 2004 Jun 26;328(7455):1535-6.
54. MSAC -MSAC. Antenatal Screening for heritable thrombophilia. Government Report. Canberra: Commonwealth Department of Health and Ageing; 2002 Aug 2002 Contract No.: Document Number].

55. Dekker JW, Lind J, Bloemenkamp KW, Quint WG, Kuijpers JC, van Doorn LJ, et al. Inherited risk of thrombosis of the fetus and intrauterine fetal death. *Eur J Obstet Gynecol Reprod Biol.* 2004 Nov 10;117(1):45-8.
56. Alonso A, Soto I, Urgelles MF, Corte JR, Rodriguez MJ, Pinto CR. Acquired and inherited thrombophilia in women with unexplained fetal losses. *Am J Obstet Gynecol.* 2002 Nov;187(5):1337-42.
57. Alfirevic Z, Roberts D, Martlew V. How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. *Eur J Obstet Gynecol Reprod Biol.* 2002 Feb 10;101(1):6-14.
58. Saade GR, McLintock C. Inherited thrombophilia and stillbirth. *Semin Perinatol.* 2002 Feb;26(1):51-69.
59. McLintock LA, Jordanides NE, Allan EK, Copland M, Stewart K, Parker A, et al. The use of a risk group stratification in the management of invasive fungal infection: a prospective validation. *Br J Haematol.* 2004 Feb;124(3):403-4.
60. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet.* 2003 Mar 15;361(9361):901-8.
61. Dudding TE, Attia J. The association between adverse pregnancy outcomes and maternal factor V Leiden genotype: a meta-analysis. *Thromb Haemost.* 2004 Apr;91(4):700-11.
62. 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.
63. McLintock C. Inherited thrombophilias: Implications for pregnancy associated venous thromboembolism and obstetric complications (Updated). Personal communication. 2004.
64. Kujovich JL. Thrombophilia and pregnancy complications. *Am J Obstet Gynecol.* 2004 Aug;191(2):412-24.
65. Raffles A, Ropel C. Non-invasive investigations are also helpful if permission for a necropsy is refused. *BMJ.* 1995 April 1, 1995;310(6983):870b-.
66. 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 Contract No.: Document Number].
67. Huisman TA. Magnetic resonance imaging: an alternative to autopsy in neonatal death? *Semin Neonatol.* 2004 Aug;9(4):347-53.

## Stillbirth investigations algorithm



## Section 5; Appendix 2 Estimation of severity of feto-maternal haemorrhage

To determine if a positive test for FMH should be considered as the likely cause of fetal death, the *percent of total fetal blood volume lost* should be calculated. Such a calculation uses the following correction factors: fetal red cells are 122% the size of adult red blood cells; 92% of fetal red cells are detected by the Kleihauer-Betke test on average; maternal red cell volume near term averages about 1800 ml; average fetal hematocrit is about 50%; fetal blood volume is about 150 ml per kilogram of body weight. Combining all of these then means that:

$$\text{Percent Fetal Blood} = \frac{\text{Fetal Cells} \times 1800 \times 1.22 \times 100}{\text{Volume Lost Maternal Cells} \times 92 \times 2 \times 100} \\ 150 \times \text{fetal wt in kg}$$

Or, to simplify,

$$\text{Percent Fetal Blood} = \frac{\text{Fetal Cells} \times 3200}{\text{Volume Lost Maternal Cells in kg} \times \text{fetal wt}}$$

So, for example, if the Kleihauer-Betke shows that 200 of 5000 cells counted are fetal and the fetus weighs 2.0 kg, then the estimate of percent blood volume loss would be:

$$200/4800 \times 3200 \div 2.0, \text{ or } 66\%.$$

Probably less than 20% volume loss is enough to cause death if it happens all at once. On the other hand, much larger volumes can be lost over a long period and the fetus can compensate. Unfortunately there is no straightforward way to know whether one is dealing with acute or chronic haemorrhage. This makes determination of whether a haemorrhage is or is not causal more problematic.

Taken from **Fetal-Maternal Hemorrhage and Stillbirth**

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