

Prenatal Diagnosis for Inherited Metabolic Disease

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Prenatal screening versus diagnosis

The purpose of prenatal testing is to detect abnormalities in the foetus so that intervention can take place involving either treatment or a termination of pregnancy.

It is important however to distinguish between prenatal (or antenatal) screening and prenatal diagnosis.

Prenatal screening such as the triple test for Downs Syndrome or ultrasound scanning is aimed at relatively low risk individuals, uses relatively invasive very low risk procedures and it is accepted that there may be some false positive and false negative results.

With prenatal diagnosis patients who have a very high risk of an affected foetus are tested using procedures with small but significant risks and a high level of reliability is required for the diagnostic test.

More recently, with advancing technology, the use of foetal DNA in maternal serum offers a less invasive test in some instances.



The Aims of Prenatal Diagnosis

- 1 To provide a range of informed choice to a couple at risk of having a child with a disease or abnormality.
- 2 To allow parents at risk to begin a pregnancy with the knowledge that the presence or absence of the disorder can be confirmed by testing.
- 3 To provide reassurance and reduce anxiety in high-risk couples.
- 4 To allow the parents to decide whether to continue the pregnancy or to terminate an affected foetus.
- 5 To allow couples to prepare for the birth of a child with health problems or a disability, or the risk of a still birth.
- 6 To enable timely medical or surgical treatment before or -- after birth.

Indications for Prenatal Diagnosis

If the mother has had a child with a severe or debilitating genetic disorder in the past that can be tested for in the foetus, e.g. inherited metabolic disorder, chromosome abnormality.

To determine foetal sex if the mother carries an X-linked disorder such as muscular dystrophy or Menkes disease.

If there is a family history of a genetic disorder for which a test can be performed.

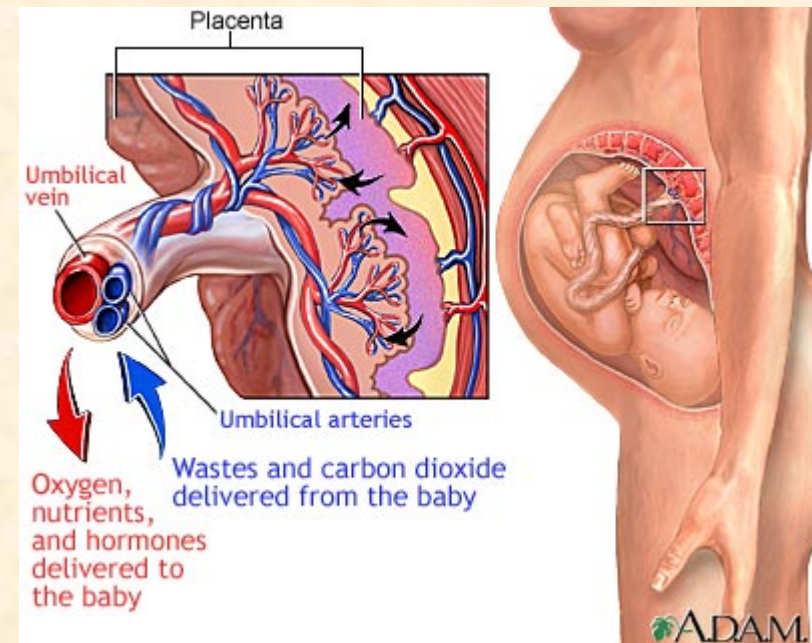
If a previous screening test (eg ultrasound, triple test) shows an increased risk for a disorder such as Down syndrome or a neural tube defect.

Requirements for Prenatal Diagnosis

- 1 The couple must be offered appropriate genetic counselling to inform them of all options available to them such as the risks involved, accuracy of the testing, potential treatments for an affected foetus.
- 2 The disease must be confirmed.
- 3 The precise defect must have been identified in the index case, either at the biochemical, enzymological or DNA level.
- 4 Both parents should ideally be tested prior to offering any testing.
Some enzymes can show extremely low levels of activity in apparently unaffected individuals - in such instances the enzyme can not be used for antenatal testing.
If DNA testing is to be used the mutation in both parents must be defined, or the affected chromosome identified if linkage is used.

Chorionic Villous Sampling

At an early stage the embryo divides into two parts, one developing into the foetus and the other into the placenta. In order to maximise contact with the maternal blood vessels finger-like formations, chorionic villi, spread out into the wall of the womb. These villi have exactly the same DNA as the developing embryo.

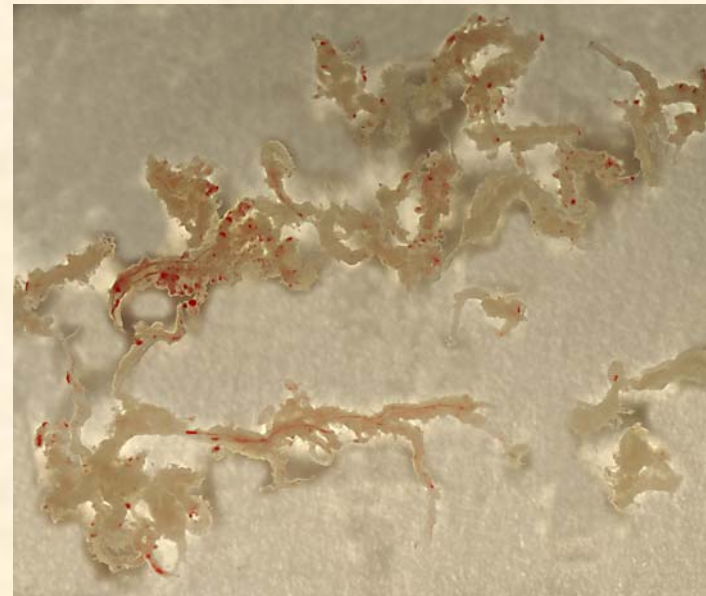
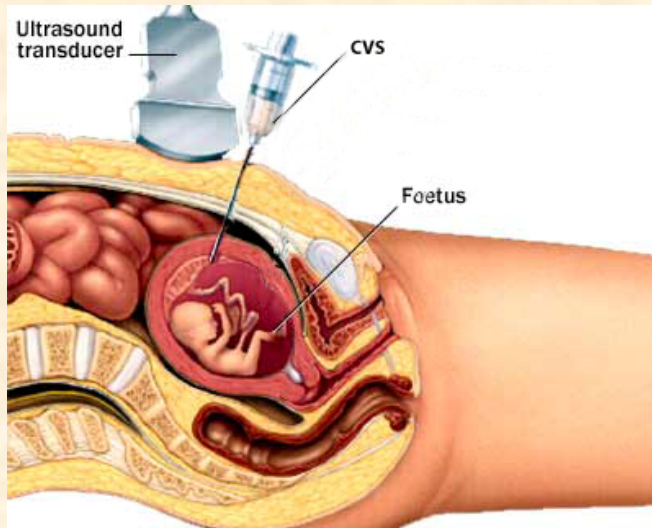


Sampling Techniques and Sample Types

Chorionic Villous Sampling

CVS is usually performed in the first trimester of pregnancy at 10-12 weeks.

Under the guidance of ultrasound scanning a small sample of the CVS is obtained either by passing a needle through the wall of the abdomen (transabdominal route) or by passing a small tube through the vagina and neck of the womb (transcervical route).



All maternal tissue has to be dissected from the foetal tissue. The tissue (see above right) can then be used directly to test for a number of metabolic disorders either by enzyme, DNA or in a few cases metabolite analysis. With this technique a results can be available in a few days and, if affected, a suction termination performed immediately.

Cells may also be cultured from the biopsy for analysis either as a first line test or to confirm the earlier results of the uncultured sample.

Risks and complications of CVS

All procedures have their risks and foetal sampling is no exception. There is a risk of approximately 1-2% of foetal loss compared to the normal foetal loss rate at this gestation and a slightly increased risk of foetal malformations.

Some enzyme tests may require a relatively large sample size (20-30 mg) compared to DNA testing (~5 mg) and in some cases it may be difficult to safely obtain a large sample. Another problem is that it can sometimes be difficult to totally remove all the maternal tissue from the biopsy. This maternal cell contamination can result in a false negative diagnosis either in analysis of the uncultured biopsy or in the cells grown from it.

Prenatal diagnosis in the case of twins or triplets is particularly difficult and care has to be taken to identify which foetuses have been sampled.

Amniotic Fluid

Amniotic fluid performs many functions, including:

- 1 Allowing the foetus to move and enabling the skeleton and lungs to develop
- 2 Maintaining a constant temperature around the foetus
- 3 Protecting the foetus by cushioning it from outside injury

By full term (40 weeks) there is approximately 600ml of amniotic fluid

Amniotic fluid (AF) contains waste products and cells shed from the foetal skin, lungs and urinary tract.

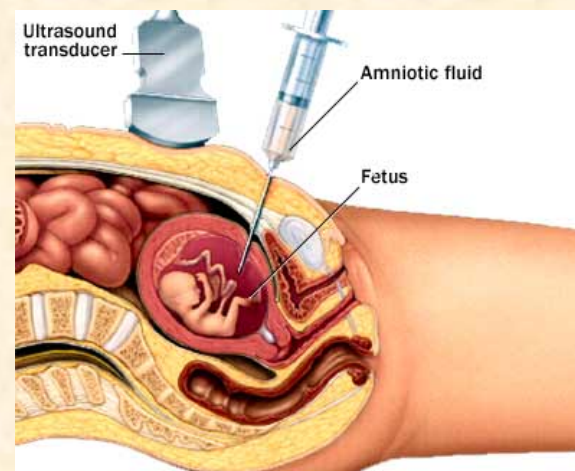
The fluid is constantly circulated by the baby swallowing and then urinating out the fluid.

It can therefore be used as a source of cells from the foetus for analysis or measurement of some metabolites excreted into the fluid.

Amniocentesis

Amniocentesis is usually performed in the second trimester of pregnancy at 14-18 weeks. It can be done earlier but there is less fluid present to sample and the procedure carries a higher risk of miscarriage (~7%)

An ultrasound is carried out to determine the best place to remove amniotic fluid from the womb. A thin needle is inserted through the abdomen and uterine walls into the amniotic fluid. Up to 10-20ml of fluid is then drawn off.



This fluid can be used to test for a number of metabolic disorders

- 1 In the cell free amniotic fluid by analysis of metabolites.
- 2 In cultured foetal cells by enzyme or DNA analysis.
- 3 In uncultured cells by DNA analysis for some disorders.

Which samples can be used and what type of tests can be done varies from disorder to disorder.

Risks and complications of amniocentesis

There is a risk of approximately 0.5% - 1% of foetal loss above the normal miscarriage risk at this gestation.

There is a small risk of a false negative result due to maternal cell contamination but this is substantially lower than CVS.

Because most tests require a cell culture step there is a risk of culture failure or the contamination of the cultures by microorganisms. Furthermore because of the time needed to culture sufficient cells for analysis (2-6 weeks) and the late sampling the result will not be available until late in the pregnancy. This can result in a high level of anxiety and, if affected, an induced rather than a suction termination of pregnancy.

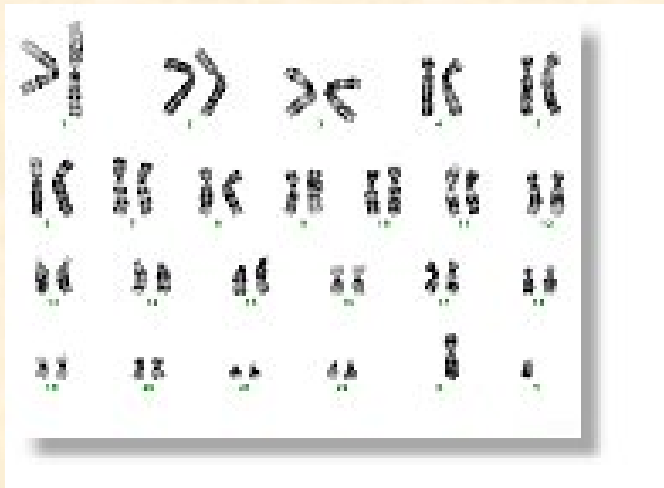
As a consequence generally CVS is preferred to amniocentesis where possible and the parents are asked to contact the appropriate clinician as early as possible when it is known the mother is pregnant.

Foetal Sexing

Where the mother is a known carrier or has a family history of an X-linked disorder the sex of the foetus may be determined as a first step.

If the foetus is female then no further testing is necessary.

If the foetus is male then further testing may be undertaken to see if he is affected.



Sexing by ultrasound can only be done reliably in the second trimester therefore a CVS is usually performed and the chromosomes analysed by karyotyping or by DNA analysis.

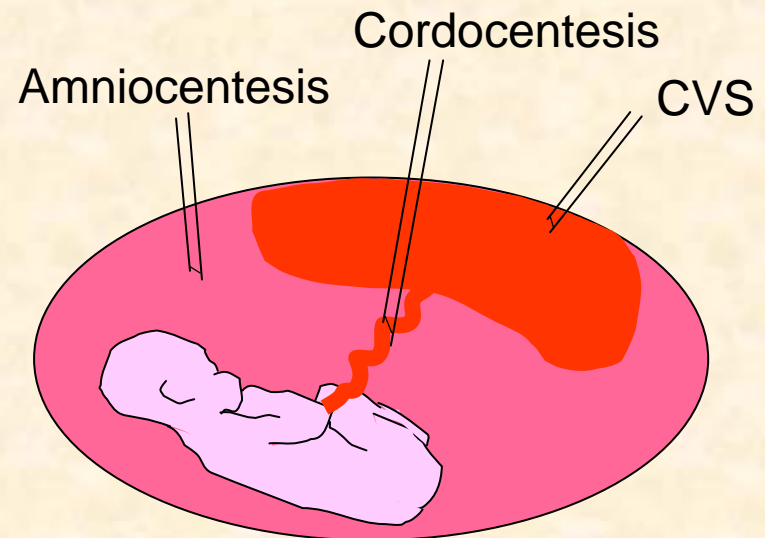
The use of foetal free DNA (ffDNA) has recently been developed. Whilst technically challenging, this procedure has the advantages that it is non-invasive (only the mother's blood needs to be tested) and can be done as early as 6 or 7 weeks into the pregnancy.

Foetal Blood Sampling

A blood sample from the foetus may be taken to diagnose a genetic disorder where CVS or amniocentesis have failed to give a clear result, to check for and treat severe foetal anaemia or Rhesus disease or to check for foetal oxygen levels or infection

It is performed after 18 weeks and can be taken from the blood vessels of the umbilical cord (cordocentesis) or from a foetal blood vessel in the heart or liver.

It is a very complex procedure and normally only done when other tests or procedures are not possible or effective and increases the risk of miscarriage by 2-3%.



Tests Available

Test Selection

Prenatal diagnosis for metabolic disease can be done by metabolite, enzyme or DNA tests on CVS, cultured CVS cells, cell-free amniotic fluid or cultured amniotic fluid cells. Which options are available and their relative reliability will vary from disorder to disorder and may change with time as experience is gained with their use and new techniques become available.

The diagnosis must have been confirmed in the index case.

DNA analysis can only be used where the causative gene has been established.

The window within which CVS is done is fairly broad but for safety reasons it is not done under 10 weeks gestation and has been done up to 14 weeks gestation. Sampling may be possible over 14 weeks but usually if it cannot be done before this time amniocentesis is the preferred option

Metabolite testing for prenatal diagnosis

A metabolic block will lead to the build up or decrease of particular metabolites within a pathway.

In many cases the foetus will exchange these molecules with the amniotic fluid such that a defect can be detected by measuring that metabolite in the cell free amniotic fluid.

This analysis is particularly useful for the organic acidaemias. The assay is often backed up by analysis of cultured amniotic fluid cells, provided the enzyme defect is known.

It is important to establish reference ranges for different gestational ages as these can vary significantly.

Enzyme analysis in prenatal diagnosis

Measurement of a defective enzyme can be carried out in cells taken by CVS or amniocentesis. In some cases the assay can be done directly on the uncultured CVS, although this sometimes requires a relatively large sample size (20-30mg) but means a result can be obtained within a few days.

In other cases the CVS or AF cells may need to be cultured before they can be analysed, meaning results could take 2-4 weeks.

Problems that can occur include:-

Some enzymes are not expressed in cultured cells whereas some assays may only be available in cultured cells.

Prior to offering any enzyme analysis the defect must be determined in the index case. Some patients may have only moderately low levels which can make an antenatal result difficult to interpret because of problems of distinguishing heterozygous from homozygous foetuses.

Some enzymes can also show extremely low levels (~10%) in healthy persons. This so called pseudodeficiency state can be an artefact of the *in vitro* assay or a genuine low level. Therefore the parents should also be tested to exclude this and also to assess what the levels of activity are in heterozygotes in that family.

In such cases antenatal testing can not be offered as a low result could be found in an affected foetus or a pseudodeficient but unaffected foetus.

Contamination of the sample with maternal cells can cause problems, particularly in cultured CVS cells.

DNA testing in prenatal diagnosis

DNA testing has the advantage that a relatively smaller sample is required than for enzymatic analysis and is generally more accurate and reliable.

However DNA analysis can only be used when both disease-causing mutations have been identified in the index case and both parents are confirmed as carriers. Alternatively linkage analysis can be used where the disease causing chromosomes are identified.

Often this requires a lot of work, particularly in disorders where more than one gene can cause the disease. As such DNA testing is not always carried out, particularly where the mother is already pregnant.

In DNA testing it is very important to exclude the presence of any contaminating DNA from the mother. Thus maternal cell contamination needs to be excluded, particularly where the result is heterozygous for the maternal mutation.

Testing for diseases of the mitochondrial DNA (mtDNA) are not routinely done as the mixture of mutant and normal mtDNA molecules can vary widely between tissues and it is not possible to predict what level the foetus might inherit.

Free foetal DNA (ffDNA)

DNA from the fetus can be detected in the blood of pregnant women and in principle can be used to test for foetal sex, Rhesus status, the inheritance of paternal mutations and some chromosome abnormalities eg Down's Syndrome.

However there are technical difficulties:

The amount of circulating foetal DNA is low (2-4% at 11-17 weeks). This can be enriched by taking advantage of the fact it is smaller (<300bp) than maternal DNA.

Appropriate measures need to be taken to ensure it is the foetal DNA that is tested and not the mother's.

In the case of inherited metabolic disease its current use is solely as a non-invasive method to determine foetal sex in the sex-linked conditions.

Options following an abnormal result

Following the result of an affected pregnancy the family may have different options.

- 1 Termination of pregnancy. Where there is no effective treatment or cure the parents may choose to abort the foetus.
- 2 Early treatment. In some disorders treatment may be available and the earlier it is started, perhaps even in utero, the better the chance for the new baby.
- 3 To prepare for the birth of an affected child. Whilst there may be no treatment available, the parents may still wish to continue with a pregnancy. The knowledge of the antenatal test allows the family to make any necessary preparations for the birth of an affected child.

Examples of the Different Tests Available for Inherited Metabolic Disorders

Non-Ketotic Hyperglycinaemia

Classically this disorder presents in the first few days of life with seizures and the baby often requires support on a respirator. CSF, plasma and urine glycine concentrations are grossly elevated. There is no treatment or cure and children often die within a few months of age. It is caused by a defect in the glycine cleavage enzyme system (GCS) which is expressed in liver, kidney, brain and placenta but not in cultured cells.

Enzyme activity can be measured in uncultured CVS but not in cultured CVS or amniotic fluid cells. Cell free amniotic fluid glycine concentrations are not significantly elevated.

DNA analysis can be performed on uncultured or cultured cells but is complicated by the fact the GCS is composed of 4 subunits, 3 of which are unique therefore more than one gene may need to be analysed.

Pyruvate Dehydrogenase (PDH) Deficiency

Severe cases of this disorder present with psychomotor retardation, hypotonia, epilepsy, ataxia and a progressive encephalopathy. CSF and blood lactate concentrations are elevated. Therapy is not usually very effective and severe cases often die within a year.

The PDH complex, which is responsible for the breakdown of pyruvate to acetyl CoA, is deficient and can be measured in cultured cells (either CVS or AF).

However an earlier diagnosis can be reached if a mutation can be defined in the affected gene. This is complicated by the fact the PDH complex is composed of 4 components, meaning more than one gene may need to be analysed. The most common defect is in the X-linked E1a subunit so if this is known to be the defective gene the sex of the foetus should be determined first.

Metachromatic Leucodystrophy

In the late infantile form children present with progressive neurodegeneration, difficulty in walking and developmental delay. Bone marrow transplant may be beneficial in some cases but needs to be started as early as possible, before any irreversible brain damage occurs. It is a lysosomal storage disorder due to a defect in the enzyme arylsulphatase A (ASA) which degrades cerebroside sulphates. The enzyme is expressed in all tissues and cell types used in antenatal diagnosis so enzyme activity can therefore be measured in uncultured cells.

However, enzyme analysis is complicated by two factors:

- 1 There is a pseudodeficiency state which exists in approximately 1-2% of the population who can have levels of activity as low as 10% with no symptoms. In these cases the enzyme level can not be used as a diagnostic tool
- 2 Extreme care has to be taken to minimize the activity of other arylsulphatases in the sample to avoid a false negative result.
- 3 The spectrophotometric assay commonly in use is relatively insensitive and levels of activity in CVS are very low even in unaffected individuals.

Because of these factors generally DNA analysis is preferred to enzyme analysis for the antenatal diagnosis of this disorder.

Menkes Disease

Onset typically begins during infancy and symptoms include hypotonia, seizures, mental retardation and developmental delay. The disorder is an X-linked recessive condition and male patients often have sparse, brittle hair. The disorder is due to a defect of copper transport across the gut and cell membranes leading to a copper deficiency state in some tissues and low serum copper concentrations. Treatment with copper supplementation may be beneficial in milder cases but otherwise the disorder is fatal. Copper concentrations in uncultured CVS can be measured but great care has to be taken to avoid copper contamination. Similarly copper abnormalities can be shown in cultured amniotic cells but they are not sufficiently reliable for antenatal diagnosis. There is no specific assay to measure function of the copper transporter.

Because of these problems DNA analysis in uncultured CVS is the method of choice by analysis of the X-linked ATP7A gene

Sex determination of the foetus is the first step and if a male foetus is predicted mutation analysis or linkage analysis can be performed (provided the mutation has been defined or the family is informative for linked markers)

Tyrosinaemia Type 1

The acute neonatal onset form of the disorder presents with liver failure, vomiting, septicaemia and hypoglycaemia. Treatment with the homogentisic acid oxidase inhibitor NTBC can be very effective.

It is due to a defect in the enzyme fumarylacetoacetatase (FAH) which is involved in the catabolism of tyrosine leading to the accumulation of the toxic compound succinylacetone and elevated plasma tyrosine concentrations.

Succinylacetone accumulates in the cell-free amniotic fluid and this can be used to diagnose the disorder in the second trimester.

The enzyme activity can be measured in uncultured CVS but the level of activity is low even in unaffected foetuses and the method relatively insensitive so very large sample sizes of uncultured CVS are required and large quantities of cells must be cultured delaying diagnosis. Also a pseudodeficiency state exists which has to be excluded in the parents and index case before enzyme testing can be offered.

DNA analysis is preferred if the mutation has been defined.

Fortunately there are a number of common mutations some specific to certain ethnic groups but full sequencing of the FAH gene can also be undertaken if required..

Morquio's Disease (MPS IV)

This progressive disorder is usually obvious between 1 to 3 years of age with short-trunk dwarfism and severe skeletal dysplasia with subsequent effects on the central nervous involvement.

Currently there is no treatment although enzyme replacement therapy is ~~in~~ being developed.

Morquio's disease is a lysosomal storage disease due to a defect in mucopolysaccharide metabolism.

It is due to defects in either the galactosamine 6-sulphatase gene or in rare cases a beta-galactosidase gene. It leads to the accumulation of keratan sulphate in urine which can be detected by urine mucopolysaccharide analysis.

Cell-free amniotic fluid does accumulate keratan sulphate but this test~~s~~ is not sufficiently reliable for antenatal diagnosis. The enzymes are widely expressed and measurement of the relevant enzyme activity in uncultured CVS or cultured CVS or amniotic fluid cells is a reliable test, once the defective enzyme has been identified in the index case.

DNA analysis can be also used if the mutations have been identified.

Methylmalonic Acidaemia (MMA)

MMA in the most severe form usually presents in the newborn with progressive encephalopathy and secondary hyperammonaemia, leading to neurological problems and death if untreated.

It is due to a defect in the breakdown of branched chain amino acids such as valine and isoleucine and some fats due to defects in a variety of enzymes involved in methylmalonic acid catabolism.

Some forms are responsive to vitamin B12 and in such cases this can be given to the mother during pregnancy. In other cases a low protein diet can help reduce attacks.

Several enzyme defects can lead to this condition and delineating which enzymes are defective and assaying them can be difficult and laborious although the assay of the incorporation of radioactive propionic acid in cultured cells can be used to detect all the disorders.

Generally the easiest and simplest way is to measure metabolites such as methylmalonic acid in cell-free amniotic fluid although this is usually only done in the second trimester.

The results can be confirmed on the cultured amniotic fluid cells using the propionate incorporation assay.

However for an early diagnosis the gene affected will have to be defined and a mutation found.

Questions

For the following disorders which would be the most suitable method for antenatal diagnosis, assuming NO DNA work has been done. NB Some disorders may have no reliable options!

Answers on the next page.

	Uncultured CVS	Cultured CVS	Amniotic fluid	Cultured AF cells
Non-Ketotic Hyperglycinaemia				
Pyruvate Dehydrogenase Deficiency				
Methylmalonic Acidaemia				
Metachromatic Leucodystrophy				
Morquio's Disease				
Menkes Disease				
Tyrosinaemia Type and mother is 16 weeks pregnant				
Tyrosinaemia type 1 and on religious grounds the couple would want any termination before 12 weeks				
Tyrosinaemia type 1 and enzyme has not been measured in index case				

	Uncultured CVS	Cultured CVS	Amniotic fluid	Cultured AF cells
Non-Ketotic Hyperglycinaemia	X			
Pyruvate Dehydrogenase Deficiency		X		X
Methylmalonic Acidaemia			X	
Metachromatic Leucodystrophy				
Morquio's Disease	X			
Menkes Disease				
Tyrosinaemia Type 1 and mother is 16 weeks pregnant			X	
Tyrosinaemia type 1 and on religious ground the couple would want any termination before 12 weeks	X			
Tyrosinaemia type 1 and enzyme has not been measured in index case			X	

Notes

Non-Ketotic Hyperglycinaemia	This is the only option
Pyruvate Dehydrogenase Deficiency	Cultured AF cells could be used but this would give a much later result
Methylmalonic Acidaemia	Metabolite measurement in the cell-free amniotic fluid is the simplest method
Metachromatic Leucodystrophy	The enzyme assay has limited reliability but may be offered subject to an explanation of the risks.
Morquio's Disease	The enzyme is much more reliable than cell-free amniotic fluid mucopolysaccharide analysis.
Menkes Disease	Copper levels can be measured but are very unreliable. DNA would be the only way- if the foetus was female there would be no need for further testing
Tyrosinaemia Type and mother is 16 weeks pregnant	This is too late for a CVS therefore succinylacetone measurement in AF is the only option, with the result being backed up on cultured AF cells by enzyme assay
Tyrosinaemia type 1 and on religious ground the couple would want any termination before 12 weeks	In order to terminate this early the enzyme assay would have to be done on uncultured CVS
Tyrosinaemia type 1 and enzyme has not been measured in index case	Although the enzyme assay could be used, the level in the index case is not known and a pseudodeficiency state could not be excluded