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Guidelines for DNA banking

Report of the Clinical Genetics Society working party on DNA banking

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1.1 Background

The Clinical Genetics Society working party on DNA banking was set up after an informal meeting of representatives of Regional Genetics Services sponsored by the Muscular Dystrophy Group of Great Britain, which took place in London on 20 November 1987. We wish to thank the Muscular Dystrophy Group for promoting debate on this important issue. We are also grateful to all our colleagues who have made valuable comments on earlier drafts of this document.

1.2 Purpose of these guidelines

These are draft guidelines for DNA banking intended to stimulate discussion and encourage review and harmonisation of laboratory procedures. The emphasis here is on practicalities. Medicolegal issues, while important, have not been considered. It is recognised that many laboratories could not implement these recommendations at present levels of funding.

2.1 Purpose of a DNA bank

DNA banks are needed to provide for the future requirements of families affected by serious single gene disorders and who require DNA analysis for the purposes of: (1) Confirmation of diagnosis at the molecular level. (2) Presymptomatic diagnosis. (3) Carrier detection. (4) Prenatal diagnosis. In addition, DNA banks constitute a valuable resource for research into the molecular pathology of genetic disorders.

2.2 Which disorders?

DNA banking should be available for any single gene disorder for which there is a consensus among professionals providing banking services that DNA analysis should be provided for the indications listed above. This applies not only to mapped genes but also to those which have yet to be localised. Commoner disorders for which DNA banking is appropriate would include cystic fibrosis, Huntington's disease, myotonic dystrophy, neurofibromatosis, tuberous sclerosis, osteogenesis imperfecta, adult polycystic kidney disease, familial adenomatous polyposis, Duchenne/Becker muscular dystrophy, X linked immunodeficiencies, and haemophilia A and B. In addition, there may be a case for banking DNA from families affected by conditions that are not single gene disorders but where a strong oligogenic component to the aetiology is suspected.

2.3 Who should do the work?

Regional Genetics Services are probably the commonest route by which families gain access to DNA testing and DNA banking should become an integral part of their work. Many centres are already storing DNA and a few have formalised arrangements which would constitute a DNA bank. In some regions other NHS units have become involved, usually with regard to one or two disorders relevant to a particular specialty. Universities and other research institutions, including several MRC units, are also collecting and storing DNA for research.

2.4 Which family members should have DNA stored?

Advice on the appropriateness of DNA banking and guidance as to which family members should have DNA stored should be sought from the centre providing DNA banking, usually the Regional Genetics Service. The common situations are as follows.

(1) To allow for molecular diagnosis and characterisation of the mutation should this become

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available in the future, DNA needs to be stored from affected family members. If none of the affected members of the family is available, it may be appropriate to store DNA from a fetus after termination of a pregnancy at risk, or from a stillbirth or neonatal death at risk for the disorder, especially in the case of X linked recessive conditions.

(2) To allow for presymptomatic diagnosis, carrier detection, or prenatal diagnosis by family linkage analysis, DNA needs to be stored from family members who are essential for determining the linkage phase. For example, given the availability of suitable polymorphic markers:

(i) Linkage phase can usually be determined for a person with an autosomal dominant disorder if DNA is available from an affected parent, unaffected parent, or preferably both.

(ii) For autosomal recessive disorders, the likely phase in the parents can usually be determined if DNA is available from their affected child, provided the DNA markers are closely linked.

(iii) For X linked recessive disorders, for a carrier woman who has an affected father or carrier mother, linkage phase can be determined if DNA is available from her father or preferably both of her parents. The phase can often be determined if only her mother is available, but this may involve more work. Alternatively it is possible to determine the likely phase in a carrier mother if DNA is available from her affected son, provided the DNA markers are closely linked.

(3) Family members who have DNA taken for analysis to meet present counselling needs will often have spare DNA banked for the future.

For an increasing number of disorders there is an urgent need to store DNA from elderly members of the family or affected persons whose life expectancy is reduced. Regional Genetics Services have a key role in the ascertainment of such persons, by (i) recall and review of families previously seen, and (ii) bringing the problem to the attention of other health professionals. Societies representing patients and families with inherited disorders can also play their part by promoting understanding of the new developments and encouraging their members to seek medical advice if DNA testing is wanted now or in the future.

2.5 Service or research or both?

Most research involving family studies for gene mapping of serious disorders will sooner or later acquire a service dimension. Researchers therefore have obligations to the families they are studying.

(1) Proper documentation should be kept and made available to whoever will be providing for the service needs of the families. The appropriate regional centre(s) should be notified that DNA is being stored (see section 4.7).

(2) In the case of elderly members of the family or affected persons whose life expectancy is reduced, there should be a clear policy about how much sample can be used for research and how much set aside for future service needs. Research workers with limited facilities could deposit an aliquot of DNA plus documentation with the appropriate Regional Genetics Service.

(3) At the end of the research project it is particularly important that proper consideration is given to the future service needs of the families.

Grant awarding bodies could demand as a condition of funding that these obligations are met.

2.6 Consent

For service work most centres do not currently obtain formal verbal or written consent before blood samples are taken for DNA analysis and banking. However, the purpose of the investigation is usually fully explained and only those who choose to be tested are sampled.

For research purposes there is a need for verbal consent and in some circumstances written consent is advisable, predictive testing for Huntington's chorea being one such instance.

3.1 Type of sample

Venous blood is currently the usual and most convenient choice. A quantity of 20 ml (preferably split into two 10 ml aliquots) should be adequate for routine purposes, providing about 800 μg of DNA. EDTA anticoagulant is cheap and convenient from the point of view of DNA extraction. Other anticoagulants could be used. It is more difficult to extract adequate amounts of DNA from clotted blood and yields are often low. Glass containers should be avoided, being unsuitable for freezing.

At necropsy, liver is a rich source of DNA if fresh, but undergoes rapid autolysis, so that beyond 24 hours after death, even with storage at 4°C, spleen is the better choice.

The new technique of polymerase chain reaction facilitates analysis of extremely small amounts of DNA and could profoundly alter the nature of DNA diagnostic work. If this technique fulfils its promise, samples suitable for DNA banking might include

dried blood spots from a Guthrie card, a few hair roots, or the buccal cells from a mouth wash.

3.2 Transport

Blood kept at ambient temperatures for a few days during transport gives adequate DNA yields. We are not aware of any systematic data relating duration, temperature, DNA yield, and integrity. Since repeated freezing and thawing of blood may prejudice DNA yield and integrity, we would advise against the freezing of blood samples before despatch since precautions then have to be taken to prevent thawing in transit.

3.3 Storage as blood

If immediate DNA analysis is not required, the sample may be stored initially as whole blood. Again, we are not aware of any systematic data relating duration of storage, temperature, DNA yield, and DNA integrity. In our experience good DNA yields have been obtained from blood samples stored at −20°C for up to two years. Storage at −70°C may be preferable, if for no other reason than in the event of freezer failure it takes longer to warm up. Repeated freezing and thawing of blood may prejudice DNA yield and integrity.

Long term storage of whole blood would not be advisable for the following reasons.

(1) The yield of DNA from whole blood samples is variable and can sometimes be very low or occasionally zero. In this event, if extraction is carried out promptly it is usually possible to obtain a repeat sample, whereas this may not be an option if processing is delayed.

(2) Blood is bulky to store and samples may come in a variety of different sized containers, which hinders efficient storage.

(3) Blood is more vulnerable to freezer failure than DNA because freezing and thawing can cause degradation.

(4) There is concern that DNA yield may be reduced if blood is stored for long periods although this has not been our experience with samples stored for one to two years.

3.4 DNA extraction

If 20 ml of blood is available, it may be preferable to extract 10 ml aliquots in separate batches to guard against mishap. After extraction, the yield of DNA in μg should be measured. In addition, it would be desirable to monitor the integrity of the DNA in all or at least some of the samples by running 1 μg on a minigel, staining with ethidium bromide, and inspecting under ultraviolet light. Presence of a dense band at the top of the gel confirms high molecular weight DNA. This could be photographed for the records. Some laboratories may wish to check the DNA for digestibility, but this involves considerably more work. Some form of quality control is particularly important in a newly established laboratory that is initially only offering facilities for DNA extraction and banking.

DNA extracted as described above is unsuitable for analysis by pulsed field gel electrophoresis. If this becomes an important diagnostic technique, it may be necessary to store some DNA in solid phase. An alternative would be to establish immortalised cell lines, but only a minority of laboratories have the facilities or funds to do this.

3.5 Storage of DNA

We would recommend that the DNA extracted from each 10 ml aliquot of blood is stored separately. This could be useful if any question arises about mislabelling of samples during DNA extraction. Opinions vary as to the best arrangements for long term storage of DNA. One option is storage in low salt buffer containing a small amount of chloroform in a sealed tube at 4°C. An alternative is storage of the bulk of the DNA at −20°C, or better at −70°C, either in solution or as a dried sample after alcohol precipitation and air drying. For this second option, we suggest that DNA be stored in aliquots as follows: (1) One working aliquot at 4°C. (2) One working aliquot at −20° or −70°C. (3) Additional long term storage aliquot(s) at −20°C or −70°C. There have been some doubts expressed about the long term stability of dried DNA and we recommend that studies be initiated into this.

3.6 Sample identification

Samples should be identified by means of both: (1) An index giving the location of the sample within the storage system. (2) Information written on the sample container in the form of patient details or a unique laboratory booking in number.

3.7 Freezer

Blood or DNA samples should preferably be kept separate from research samples in a designated freezer which is used solely for this purpose. Care should be taken not to store blood or DNA together with plasmid. Consideration should be given to the following.

(1) *Power failure.* DNA will tolerate some freezing

and thawing so that a single power failure does little harm. However, if this is likely to be a frequent occurrence, or if blood is being stored, consideration should be given to an alarm system and also either an emergency power supply or a liquid nitrogen back up system which floods the freezer if the power fails. Such systems require an electrical supply to operate the valve and this can be from either the emergency power circuits or accumulators.

(2) *Fire risk*. There should be an alarm system and ideally perhaps measures to render the freezer location resistant to fire. The only safeguard possible against major fire would be storage of aliquots of DNA in different parts of the building or separate buildings, neither of which may be feasible options.

(3) *Security*. Access to the freezer should be restricted to designated persons. It may be appropriate to keep the freezer locked.

If research and service samples are kept together in the same freezer, it is essential that research workers are mindful of the service needs of the families.

3.8 Monitoring usage

Sample usage should be monitored and a record kept.

3.9 Storage of filters

Filters used for current analysis should be stored for repeat analysis when appropriate. This is possible with nylon membranes which should be rinsed with TE buffer and dried. We are not aware of any data about long term storage of membranes, but would suggest they be sealed in polyethylene envelopes and stored at room temperature. Wet filters stored in polyethylene are at risk of fungal growth.

3.10 Duration of storage

DNA may need to be kept for several decades, so that DNA banking should be seen as a long term commitment with appropriate administrative arrangements and secure funding.

Opportunities may arise to discard DNA that is no longer needed for service purposes. For example, the development of direct methods for detecting a mutation in heterozygotes and homozygotes would allow the discarding of samples previously needed for linkage based analyses. Indeed, in these circumstances, once the mutation in a family had been adequately characterised, there would be no need to store DNA from any family members. There should be a carefully thought out procedure for the disposal of DNA samples, so that this is only permitted after the most careful consideration. Such samples may still be valuable for research purposes, including the mapping of new markers and studies of the origin of mutation events.

4.1 Documentation with each specimen

Samples should be submitted with a request form designed for the purpose. It is essential that sufficient details come with each specimen to identify the person sampled reliably. This should include as a minimum:

(1) Surname, first names, and date of birth on both the specimen and the request form.

(2) Name and address of person submitting the sample.

(3) Referring laboratory booking in number for the sample, if appropriate.

(4) Date of sampling.

(5) Family identification by (i) name and date of birth of the index case or nearest affected relative, and (ii) pedigree number if one has been assigned.

(6) Diagnosis.

Before proceeding with the analysis, the laboratory will also need to know the purpose of the investigation and will usually require a family pedigree, details of the diagnosis, and other information given in 4.4.

4.2 Booking in

It is essential that a foolproof booking in system is instituted and rigorously followed, so that samples are never switched. This is particularly important when samples from several members of the same family arrive at the laboratory together. We recommend that research samples are also put through the same booking in system.

4.3 Reporting

Even if no DNA analysis is carried out, it is helpful if the laboratory issues a report stating that DNA has been successfully extracted and stored. A copy could go to the general practitioner for filing in the patient's records. It would be desirable for other copies to be lodged in the general practice records of immediate relatives who may in the future request DNA studies for which this sample would be needed.

4.4 Documentation of the family

For each family the DNA bank should have access to the following data.

(1) Unique pedigree number.

(2) Family pedigree.

(3) Full identification of relevant family members, including surname, first names, date of birth, maiden name, and any previous married names, and name and address of general practitioner.

(4) Diagnosis.

(5) Summary of diagnostic criteria fulfilled for at least one affected member of the family.

(6) Summary of information relevant to assigning family members as affected, unaffected, or carrier, or for determining conditional odds.

(7) Availability of DNA.

(8) Results of DNA analyses already carried out.

(9) Availability of filters.

It would be valuable to establish a uniform data set for Regional Genetics Services and other institutions providing DNA analysis and banking.

We would emphasise the following points. (1) Careful and critical review of the diagnosis should precede any request for molecular genetic investigation. (2) Linkage studies, particularly those involving extragenic markers, should be directed by someone experienced in this type of analysis. (3) There should be close liaison between DNA banks and those advising the family. It is desirable that contact with a new family should lead to all family members at risk being offered counselling and to provision being made for the future counselling needs of the family.

4.5 Data storage and retrieval

A well thought out system for data storage and retrieval is essential. Whether manual or computerised records are used, consideration should be given to security, confidentiality, and fire hazards. For computerised records, in addition, there is the need to consider back up of stored data and to comply with the Data Protection Act.

4.6 Information retrieval: the wider problem

If a key person in a family has DNA stored and then dies, how can we ensure when the need arises years later that those advising the family will know that DNA is available and where it is banked? There are several problems to overcome.

(1) Members of the same family may reside within different Regional Health Authority areas.

(2) Families move around the country.

(3) Primary care records are destroyed when a patient dies.

(4) Hospital records may be destroyed or selectively copied onto microfilm or fiche.

4.7 Suggested solutions

We suggest the following but would welcome discussion on this difficult question.

(1) One centre in each region (usually the Regional Genetics Service) could undertake to maintain a register of all institutions in their region who are storing DNA for service or research purposes. The regional centres could then collaborate to maintain an up to date directory of all DNA banks in the United Kingdom with (i) name and address of the institution, (ii) name of a contact person, (iii) telephone number, preferably a direct line, (iv) area covered, and (v) diseases covered. The directory would be circulated to all Regional Genetics Services and other interested parties on a regular basis. Alternatively, the possibility of a central register of DNA banks could be explored, perhaps maintained on computer with on line access.

(2) Anyone storing DNA from families should be under an obligation to notify the appropriate regional centre.

(3) In response to request by letter or telephone, centres providing DNA banking services should be able to determine immediately whether a person or family are known to them by reference to a properly maintained database and also to determine whether DNA is available.

(4) Research workers should liaise fully with those responsible for providing for the current or future service needs of the families, following the recommendations of section 2.5.

(5) Reports stating that DNA has been extracted and stored should be lodged in the general practice records as outlined in section 4.3.

4.8 Other possibilities to consider

Some colleagues have argued strongly for a national register (along the lines of the National Haemophilia Register) containing the surname, first name, and date of birth of all persons who have DNA banked in the United Kingdom, together with details of the centre holding the sample. The data could be collected in two stages. One centre in each

region (usually the Regional Genetics Service) would have the responsibility of collecting the data from all the DNA banks in their region, preferably in machine readable form. This information would then be passed on to the central register. On line password controlled access to this database would be one option, but raises issues of confidentiality. Alternatively, the whole database on computer disk or other machine readable media could be distributed to contributing centres and updated at regular intervals. Establishing and maintaining a national register would require funding as well as a substantial commitment from all the regional centres and DNA banks involved. We doubt whether the benefits would justify such a major undertaking.

5 New developments

The authors recognise that as new methods, such as polymerase chain reaction, become established, some of the technical sections of this document will need revision. Other sections, particularly relating to confirmation of diagnosis, documentation, and data retrieval will remain of fundamental importance for the forseeable future.

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