Setting up a NI PD Biobank of maternal samples: Sample collection, Storage and DNA extraction protocols

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DIN ISO 9001:2000 certified
Study design

What is your target? DNA, RNA, Protein, Cells, Plasma? Size estimate
Example 1: Non-invasive determination of fetal *RHD*-status from maternal plasma during pregnancy in at least 1000 cases.
Example 2: Biobank of researchers involved in NIPD

What is your reference? Are these clinical data, or do you need reference tests?
Example 1: Comparison with D-status from the newborn, determined with serology
Example 2: reports, abstracts, papers

What happens, if discrepancies are observed?
Example 1: If discrepancies were observed, final conclusions were made from repeat tests from a 2nd aliquot and buccal swap analysis of the newborn
Example 2: If discrepancies are observed, study participants will be contacted for another interview
Sample preparation and storage

If possible, separate plasma from cells within 24 hours and freeze plasma <-70°C

Two centrifugation steps
10 min 1,200xg
20 min 6,000xg
Define exclusion criteria

Example:

Hemolysis, Serum
Checklist complete?

Target population defined?

Sample size determined according to prevalence/incidence of marker?

Reference defined (cell culture, questionnaire, clinical report)?

Sample transport conditions defined?

Sample handling and storage protocol defined?

Cross-Check with clinicians positive?

Freezing capacity checked? Temperature monitored and prepared for failure of the freezer?
Ethics: Principle rules

Recommendations of the European Society of Human Genetics
European Journal of Human Genetics (2003) 11, Suppl 2, S8–S10

http://npg.nature.com/ejhg/journal/v11/n2s/pdf/5201115a.pdf

DATA STORAGE AND DNA BANKING FOR BIOMEDICAL RESEARCH: TECHNICAL, SOCIAL AND ETHICAL ISSUES

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Statements and Recommendations

Reason for DNA banking

Responsibility of proper management and protection of the subjects interests
Status of collection

**Anonymous collection**
Biological material without identifiers, impossible to link with the source

**Anonymized collection**
Biological material, originally identified, all identifiers irreversibly stripped
Demographic and clinical data attached to anonymized samples should be coded with international nomenclatures

**Identifiable collection**
Biological material, unidentified for research, can be linked to source with code

**Identified collection**
Biological material, name, patient number, pedigree location attached to material
Consent requirements for new collections

Informed consent required for all types of DNA banking

For research use, ethical approval is required

Consent

Written, specific protection for vulnerable subjects, principle of best interest
Content of Consent

Type of research (broader use), arrangements for access to or sharing of samples, duration of storage

Consent should be free from pressure, based on information provided by trained staff

Right to withdraw at any time without reason, including destruction of sample
(Anonymized collection: may be used for other purposes than those originally intended)
Existing collections

Identifiable or identified collection: Investigators should re-contact subjects to obtain consent for new studies

Anonymized

If it is impracticable to gain consent, review board should give its consent based on the notion of minimum risk for the donor

Post-mortem use of samples
Not allowed if individuals restrict use of their sample
Management, quality control and security issues

Confidentiality

Standardization of coding, sample tracking, computerization and encryption

Written protocol describing the rights and obligations of all parties with respect to storage and access data and samples

Provisions should ensure continued care of the collection under any circumstances (Follow Data Protection Act, controls on access to data, physical security)

Population studies
Consent at a group level, cultural appropriate authorities, respect minority rights
Summary

Consent, confidentiality and coding are the key principles for DNA banking.

Respect the fundamental rights of study individuals declared in the Charter of Fundamental Rights of the European Union and in the Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data.

For any project using human material specifically collected for research, the convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (Convention on Human Rights and Biomedicine) requires Ethical approval.
Recruitment strategies

Mail, e-mail to individuals

Mail, e-mail to physicians

Telephone

Free mailing of samples and data

Reimbursement of staff involved

Reimbursement of travel/parking costs for participants
Topics for Ethical Approval

Aim of the study
Current gold standard, scientific background
Study-protocol
Recruitment of study persons, inclusion, exclusion criteria
Group-size estimate (Statistics)
Protection of Material and Data
Literature
Information leaflet
Consent leaflet
DNA-Extraction Methods

• 1. Disruption

• 2. Lysis (90°C 20 min, sucrose, NH₄Cl, SDS, GuSCN, GuCl, nonionic detergents)

• 3. Removal of proteins and contaminants (typically proteinase K)

• 4. Recovery of DNA
Recovery of DNA

Salting out: Ammonium acetate or potassium acetate
Followed by alcohol precipitation
Disadvantage: Protein and RNA contamination, variable yield

Phenol/Chloroform/Isoamylalcohol 25:24:1 pH 7.5-8.0
Aqueous phase contains RNA and DNA, followed by alcohol precipitation
Disadvantage: Toxic organic solvents, multiple tube transfer, time-consuming, may inhibit PCR

Cesium chloride-ethidium bromide density gradient
Disadvantage: Requires toxic ethidium bromide, ultracentrifugation for hours, time consuming, labor intensive
DNA-Extraction Methods: Principles

Binding to solid-phase
- anion-exchange
- silica-based
- specific probes

Solid phase
- membrane
- particles
Magnetic tip

cover

Remove magnetic tip

Beads drop off
Remove cover

collect beads
large volume

remove beads

Transfer to small
wash volume
# First evaluation of cff extraction using magnetic particles

<table>
<thead>
<tr>
<th>Method</th>
<th>Manufacturer</th>
<th>Volume, extracted ($V_{ex}$)</th>
<th>Volume/PCR</th>
<th>Positive reaction (+) according to $10^6$</th>
<th>$10^5$</th>
<th>$10^4$</th>
<th>$10^3$</th>
<th>$10^2$</th>
<th>10</th>
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<tbody>
<tr>
<td>Tip-Extraktion</td>
<td></td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>-</td>
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<td>2000</td>
<td>17</td>
<td>+</td>
<td>+</td>
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<td>2400</td>
<td>86</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>n.t.</td>
</tr>
</tbody>
</table>

*Note: n.t. indicates not tested.*
Manual DNA Extraction Methods 1

QIAamp DSP Virus Kit (QIAGEN, Hilden, Germany), Cat. No. 60704

HP: High Pure PCR Template Preparation Kit, MINI: QIAamp DNA Blood Mini Kit, CST: CST genomic DNA purification Kit, MB: in-house magnetic bead separation method, MIDI: QIAamp DNA Blood Midi Kit

Manual Extraction Methods (Pool 3)

pg/well

- DSP
- HP
- MINI
- CST
- MB
- MIDI

## Automated DNA-Extraction Methods
### SAFE-NoE survey 2004

<table>
<thead>
<tr>
<th>Equipment</th>
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<tr>
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<td>Roche</td>
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<tr>
<td>1 ABI 6100</td>
<td>ABI</td>
</tr>
<tr>
<td>1 Kingfisher</td>
<td>Thermo Electron</td>
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<tr>
<td>1 Separation Module 1</td>
<td>Chemagen</td>
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<tr>
<td>1 Tip Extractor (in-house)</td>
<td>Tecan</td>
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<tr>
<td>1 Biorobot MDx</td>
<td>QIAGEN</td>
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<tr>
<td>1 Biorobot M48</td>
<td>QIAGEN</td>
</tr>
<tr>
<td>1 EZ1</td>
<td>QIAGEN</td>
</tr>
</tbody>
</table>
Automated DNA Extraction Methods

MP: Magnapure Roche, Tecan: Tip-Extraction, MDx, M48, EZ1 instruments from QIAGEN

Automated DNA Extraction Methods

Conclusion

Only the MagnaPure LC system and the robotic Magnetic Tip System reliably detected cff DNA in low concentrations.

Recently, Qiagen has developed a new pipetting procedure for the BioRobot MDx with higher sample input volume and smaller elution volume and this new option will be further evaluated in the future.
# Automated DNA-Extraction Methods

**SAFE-NoE survey 2006**

<table>
<thead>
<tr>
<th>No.</th>
<th>Instrument</th>
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<tr>
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<tr>
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<tr>
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<td>Separation Module 1</td>
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<td>Tip Extractor (in-house)</td>
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<td>3</td>
<td>Biorobot MDx</td>
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<tr>
<td>1</td>
<td>EZ1</td>
<td>QIAGEN</td>
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</tbody>
</table>
# Evaluation of 2 extraction methods

**Chemagen Magnetic Separation Module 1**  
*(Chemagen, Baesweiler, Germany)*

<table>
<thead>
<tr>
<th></th>
<th>QIAamp DSP Virus Kit</th>
<th>Chemagen Magn. Sep. Mod. 1</th>
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<tbody>
<tr>
<td>Input Plasma</td>
<td>500µl</td>
<td>1000µl</td>
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<tr>
<td>Elution volume</td>
<td>40µl</td>
<td>100µl</td>
</tr>
<tr>
<td>Elution Reagent</td>
<td>AVE</td>
<td>Elution buffer</td>
</tr>
<tr>
<td>DNA/ <em>RHD</em>-PCR</td>
<td>15µl</td>
<td>15µl</td>
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<tr>
<td>Plasma-equivalent/PCR</td>
<td>187.5µl</td>
<td>150µl</td>
</tr>
<tr>
<td>Processing time</td>
<td>~2.5h</td>
<td>~1.5h</td>
</tr>
<tr>
<td>max. samples/run</td>
<td>about 24</td>
<td>12</td>
</tr>
</tbody>
</table>
More details on this study

Sina Müller

NI PD on RHD – a feasibility study

Today 16:30-16:35
Fetal DNA Yield (*RHD* exon 7)

620 D-neg. samples with D-pos. fetus

DSP Virus Kit: 6 – 6973pg/ml, median 101pg/ml
Chemagen Magn. Sep. Mod. 1: 43 – 34.418pg/ml, median 703pg/ml
Conclusions

Cff DNA extraction from maternal plasma is now feasible and reliable.

Due to our latest knowledge, sensitivity is now about 99.8% (Chemagen MSM1) and 99.7% (QIAGEN DSP Virus Kit), respectively.

MDx might be an alternative too (comparison not done, yet)

In order to avoid maternal contamination samples should be separated and frozen within 24-32 hours
Acknowledgment

Participating obstetricians and patients
All SAFE Partners, especially
Ellen van der Schoot, Aicha Ait Soussan
Evelyn Tait, Sylvia Armstrong-Fisher, Stan Urbaniak
Ilona Hromadnikova
Sinuhe Hahn, Tea Rekhviashvili, Ying Li, Wolfgang Holzgreve

Funding from the European Commission for the SAFE-NoE (LSHB-CT-2004 503243) for which this research was partially funded is gratefully acknowledged.
Thank you for your attention!