MOLECULAR GENETICS DIAGNOSTIC LABORATORY:
DNA QUALITY TASK FORCE INVESTIGATION

Prepared by the Molecular Genetics Diagnostic Laboratory DNA Quality Task Force.

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Background

Molecular Genetics Diagnostic Laboratory

The Molecular Genetics Diagnostic Laboratory at the Children’s Hospital of Eastern Ontario (CHEO) serves as a reference laboratory for the Eastern Ontario Region and offers molecular genetic testing for 27 heritable conditions. The Molecular Genetics Diagnostic Laboratory also offers DNA banking and DNA “send-out” services, when appropriate. Upon request, DNA from clinical samples can be banked for future genetic testing or until the sample is no longer required. If a genetic test is not offered within the Molecular Genetics Diagnostic Laboratory, but an appropriate laboratory has been identified elsewhere, then arrangements can be made to have DNA “sent-out” to the external laboratory accordingly. In the year from April 2010 to March 2011 the Laboratory received 4,592 specimens for processing.

Most of the tests available through the CHEO Molecular Genetics Diagnostic Laboratory are performed on DNA extracted from peripheral blood. DNA from fibroblasts, biopsy/surgical tissues, chorionic villi and amniotic fluid may also be tested for some referrals. Upon specimen receipt DNA is extracted using either an automated or manual process depending on the type of sample received and the requirement for relatively large quantities of high molecular weight DNA in downstream testing. For genetic testing of fragile X syndrome, myotonic dystrophy (type 1 and 2), hereditary breast/ovarian cancer (BRCA1/2), hereditary non-polyposis colon cancer and cystic fibrosis (positive newborn screening referrals only) DNA is prepared from peripheral blood specimens using a commercially available kit (ArchivePure DNA Blood Kit; 5 Prime, Inc, Gaithersburg, USA). All peripheral blood samples that are received for DNA banking or DNA send-out are also extracted using the ArchivePure DNA Blood Kit. For genetic testing of fascioscapular humeral muscular dystrophy (FSHD) peripheral blood cells are embedded in agarose and DNA is manually extracted in situ. For all remaining tests available peripheral blood samples are processed using an automated DNA extraction system (NucliSENS easyMag Extractor; bioMerieux Clinical Diagnostics). Finally, for specimens other than peripheral blood, DNA is extracted using a manual, phenol-chloroform based methodology. The quality of all manually extracted DNA samples is evaluated post-extraction by measuring optical density (absorbance readings at 260 and 280 nm wavelengths) to calculate DNA concentration and DNA to protein ratios. All samples extracted manually (including all samples for DNA banking or send-out) are further evaluated on electrophoretic gels to assess DNA integrity and allow early detection of any issues related to DNA degradation.

Prior DNA Quality Investigations

The CHEO Molecular Genetics Diagnostic Laboratory strives for excellence in genetic testing and relies on the highest quality of DNA to ensure delivery of timely, sensitive and accurate testing services. Despite efforts to ensure quality in DNA extraction procedures, over the last few years the Laboratory has experienced a number of incidences which have required investigations regarding DNA quality.

In the period between February 2008 to January 2009 a number of DNA samples were identified that had been extracted and shipped by the CHEO Molecular Genetics Diagnostic Laboratory but were reported to be of insufficient quality for testing by the referral laboratory (23 of 336 DNA samples shipped during the indicated time period). Most of the test failures occurred at one of two sites. An investigation of the test failures was initiated including retrospective analysis of DNA “send-outs” to identify additional test failures, and direct measurements of DNA quality, ie. concentration, protein or organic contamination of samples, and presence of low molecular weight, degraded DNA. DNA quality measurements were compared between samples that had been successfully
and unsuccessfully tested at the external laboratory. While insufficient DNA quantity was identified as an issue for the test failures at one external site, no trends were identified to explain the observed test failures at the other site. Interestingly, in addition to reports of test failures involving DNA samples shipped from CHEO, this investigation also led to the identification of three samples shipped as blood to the second external site that likewise could not be tested; again “insufficient DNA quality” was cited as the reason for the test failure. Test failure for the samples shipped as blood rather than DNA suggested that there may have been contributing factors at the external laboratory site that at least partially led to the unsatisfactory results through this specific laboratory. Following recommendations arising from the above investigations a number of changes were implemented in the Laboratory to improve the DNA send-out service, including:

- shipment of samples to referral laboratories as blood rather than DNA whenever possible;
- use of manual DNA extraction methods for processing of blood samples with a positive newborn screen results for cystic fibrosis to ensure sufficient DNA quantities for downstream CFTR gene sequencing, if required;
- development of a reporting tool to be shipped with all DNA send-outs to encourage referral labs to notify the CHEO Molecular Genetics Laboratory if the DNA shipped was compromised or of insufficient quality;
- development of a tracking system to allow more accurate record keeping of which samples were extracted using which technology.

Subsequent to the above review there have been two other prior investigations related to DNA quality, both of which have led to important quality control and assurance improvements to the DNA extraction process in the CHEO Molecular Genetics Diagnostic Laboratory. In November 2009-March 2010 and in December 2010 significant concerns related to DNA quality were identified. A majority of the samples extracted during the relevant period had shown evidence of DNA degradation. Although the degraded DNA was sufficient for a number of downstream genetic tests (i.e. PCR-based methodologies), it was not suitable for tests requiring high molecular weight DNA (i.e. chromosomal microarray analysis or Southern blots). In the first incident the source of DNA degradation was isolated to bacterial contamination of the water supply that was used for the preparation of several reagents within the laboratory. Residual bacterial contamination of a non-autoclavable reagent pump is also thought to be the source of the second incident of degradation. The problem has since been addressed by replacing the water source used for preparation of reagents with commercially available, certified molecular grade water (Sigma-Aldrich, St. Louis, MO and BDH Chemicals, VWR International, Radnor, PA). To minimize the risk of bacterial contamination, the use of disposable tips/reagents dispensing instruments has also been introduced to all critical steps of the DNA extraction process. Electrophoretic gels were also introduced as a quality assurance measure to verify DNA integrity of samples extracted using in-house or commercial manual extraction protocols (integrity gels). The rapid identification and resolution of degradation-related issues in December 2010 is partially accredited to the prior incorporation of these integrity gels into the Laboratory quality assurance/quality control (QA/QC) program.

**DNA Quality Task Force**

Despite the above improvements to the DNA extraction process, in April 2011 the CHEO Molecular Genetics Diagnostic Laboratory was notified of two test failures involving DNA that had been extracted and shipped by the Laboratory to an external facility for testing. Concurrently there was evidence from the most recent integrity gels that a small proportion of the DNA extracts prepared by the Laboratory had been degraded. These two situations were taken very seriously, particularly in light of the prior DNA quality investigations. A task force was subsequently formed to investigate the most recent incidents of DNA degradation, and to assess whether there were changes to the DNA extraction process in the Laboratory to prevent similar incidents in the future.
The task force committee includes technical members of the Laboratory staff (Senior Technologist - Dr. Ed Yeh, Specialty Technologist - Virginia Haslett, Medical Laboratory Assistant – (Janna Poapst), Medical Laboratory Assistant – (Alicia Storey), a clinical post-doctoral fellow (Dr. Olga Jarinova), and the Laboratory Quality Coordinator (Lorraine Hart). It is chaired by the Clinical Laboratory Scientist (Dr. Elizabeth McCready) and reports to the Head of the Molecular Genetics Laboratory (Dr. Nancy Carson), the Genetics PSU Operations Director (Gabrielle Mettler), and the Department of Genetics Laboratory Director (Dr. Jean McGowan-Jordan). The task force has also been advised by an external Scientist, the director the Alberta Regional Laboratory Services – Clinical Genetics Laboratories (Dr. Martin Somerville).
Task Force Objectives and Goals

Primary Objective:

To ensure consistency in the generation of quality DNA within the Molecular Genetics Diagnostic Laboratory by establishing guidelines and recommendations related to sample receipt, DNA extraction and DNA storage.

Task Force Goals:

a. To establish sample acceptance/rejection criteria and develop related policies and procedures including issuing reports for rejected samples.

b. To examine existing DNA quality indicator data to identify trends that may influence current practice, and develop new quality indicators as required allowing suitable measurement and trending of the factors that can influence DNA quality.

c. To evaluate existing DNA extraction protocols to ensure accuracy and consistency in the performance of procedures as per relevant publications, and kit manufacturer recommended procedures.

d. To evaluate the supplies and reagents used for DNA extraction and storage procedures to ensure that all products and equipment used are being used as intended.

e. To evaluate existing QA/QC processes employed for the assessment of DNA quality and provide recommendations for modifications to these processes, if required.

f. To investigate causes of “failed clinical tests” previously reported by outside laboratories involving DNA prepared by the CHEO Molecular Diagnostic Laboratory. The results of these investigations will be evaluated to assess whether further process changes should occur that may impact DNA quality and are not otherwise addressed by the goals outlined in this document.

g. To identify a suggested notification system to enable timely reporting to Laboratory staff of ALL test failures involving DNA shipped from the Laboratory.

h. To develop a process for investigation of any future test failures involving DNA shipped from the Laboratory that ensures consistent evaluation and follow-up of each report.

i. To assess current procedures for DNA storage, and provide recommendations for the storage and appropriate disposal of DNA stored within the CHEO Molecular Diagnostic Laboratory.
Methods

Retrospective Investigation of Reported or Observed Suboptimal DNA Extracts

DNA Send-outs: Failed Genetic Tests at Referral Sites

To allow investigation of quality issues related to DNA samples shipped from the CHEO Molecular Genetics Diagnostic Laboratory, CHEO physicians and genetic counselors were asked to submit any reports/notifications received from another laboratory indicating that results were unanalyzable or that the provided DNA was of insufficient quality for testing. Relevant reports were reviewed to determine whether DNA quality or degradation was implicated in the failed test results. The DNA quality of the corresponding samples was ascertained by performing an integrity gel on the remaining sample retained by Molecular Genetics. Further investigations also included retrospective examination of Molecular Genetics patient files and log books for notes related to the extract.

Sporadic Incidence of Degraded DNA within the CHEO Molecular Genetics Diagnostic Laboratory

In an effort to isolate factors that may have contributed to sporadic incidents of suboptimal DNA quality, the task force committee reviewed integrity gels from January 2011 to June 2011 to identify individual samples that showed evidence of DNA degradation. The amount of degradation was classified as “minor”, “moderate” or “major” depending on the amount of sheared DNA visible on the gel, and the presence of high molecular weight in the sample. Samples with mostly intact DNA and minimal shearing of the high molecular weight DNA were classified as having “minor” degradation. Samples with sheared, low molecular weight DNA were classified as having “major” degradation if a band corresponding to intact high molecular weight DNA was not apparent, or “moderate” degradation if the high molecular weight DNA band was present. It should be noted that since May 2011 all samples extracted using a manual protocol are examined by electrophoresis to assess DNA integrity. Prior to this period, however, samples extracted manually for purposes other than banking or shipping were randomly selected for analysis on an integrity gel. (All banked and send-out samples were electrophoresed in both time periods). Because not every sample extracted manually was included on integrity gels the group of degraded samples identified as part of this particular retrospective study is unlikely to represent a complete list of degraded samples from the specified time period.

Having had identified a list of samples with evidence of degradation by electrophoresis, the Task Force Committee collected the following data from DNA log books, requisitions and chart communication charts to assess if there were any trends that may have contributed to the incidence of degradation.

- Date of DNA extraction
- Age of samples upon receipt
- Collection sites
- Name of the technologist/technician who performed the extraction
- Whether samples of good DNA integrity were present in the same batch with the degraded sample/samples
- Staff notes reflecting suboptimal sample conditions that may have contributed to degradation (such as “insufficient volume of blood, clotted sample etc”)
Samples for which relevant information regarding the above parameters were not available, were excluded from further analysis.

**DNA Extraction Process – Overview**

To aid task force and focus group discussions a flow chart was prepared that outlines specimen processing from sample receipt to sample storage. These flow charts were prepared in conjunction with a walk through of the Laboratory by the Senior Technologist of Molecular Diagnostics in Virology (Laura Shaw) and the Quality Coordinator-Laboratories (Lorraine Hart). L. Shaw has over 25 years experience in DNA extraction procedures and was asked by the committee to provide an objective review of the extraction process in the Molecular Genetics Laboratory to assess if there were any obvious issues that may contribute to DNA degradation. L. Hart has extensive training in quality control and quality assurance programs and in process evaluation using LEAN principles; she was asked to review the overall process and the QA/QC procedures to assess if there were process inefficiencies or required changes to the quality program. The walk through was guided by the technician who regularly performs DNA extraction and included a demonstration of each step along the route of a specimen processing. All pre-analytical, analytical and post analytical steps were examined. Process differences between the Virology and Genetics Laboratories were highlighted by L. Shaw as factors that could be considered by the task force investigations.

**DNA Quality Focus Group**

Considerable experience exists within the Genetics PSU related to clinical laboratory process, DNA extraction and molecular methodologies. As such, a focus group was organized to survey experienced technologists and other laboratory personnel regarding potential factors that may have contributed to the most recent DNA degradation issues. Opinions regarding QA/QC processes, or procedural considerations that could enhance the existing DNA service were also explored. Specifically, participants were asked to provide opinions about the following topics:

- Sample acceptance/rejection criteria
- DNA quality measurements
- DNA extraction protocols
- Extraction supplies and reagents
- DNA send-outs and follow-up of testing failures at referral laboratories
- DNA storage

Participants provided comments on note paper about aspects of each topic that either shouldn’t be changed or could be improved. Time was also provided for brief discussion about most of the topics. Following the focus group, comments were summarized in a table format, and provided to each of the participants for their review and comment. A “FISH bone” diagram of the comments was further developed to facilitate ongoing Task Force activities.
Literature Search

A review was performed to evaluate the available information in the published literature over the last 20 years or so regarding DNA extractions and factors that can influence DNA quality. This literature review was aided by the CHEO Library staff. Topics examined in the literature searches included the following:

- Acceptable peripheral blood sample age for preparation of high molecular weight DNA;
- Affect of hemolysis or clotting on preparation of high molecular weight DNA;
- Optimal methods/solutions for rehydration of extracted DNA; and
- Affect of residual alcohols from extraction processes on DNA stability.

ArchivePure Manual DNA Extraction - Protocol Refinement

To investigate whether use of the Archive Pure Blood kit could contribute to the observed incidents of DNA degradation, correspondence with the kit manufacturer, 5 Prime Inc., was initiated. The issue of DNA degradation was brought forth and discussion was focused on critical steps in the extraction procedure that could affect DNA quality. In addition, procedures in the Molecular Genetics protocol not specifically mentioned in the manufacturer’s procedure were discussed.

In addition to review of the existing protocols according to the manufacturer’s recommended procedures, performance of the extraction procedure was observed bench-side to identify possible variations in techniques between Laboratory staff members that may contribute to the sporadic nature of the observed degradation issues. Technicians/technologists performing DNA extractions were shadowed during the DNA extraction process. Staff was instructed to perform the extractions as they would normally. Detailed notes describing the actions taken by the extracting technician/technologist, as well as any comments that were offered, were documented by a single observer. The same observer was used for all shadows and took place over the course of several weeks. A review of the notes generated during the shadowing was then performed by a second individual. Differences in the performance of the protocol between users and observed deviations from the written protocol were summarized and used to assess specific lines of the approved Laboratory protocol that may require revision to clarify the protocol and avoid user-dependent variations of protocol performance.

Reagent Evaluation

In order to identify factors that may contribute to production of suboptimal DNA, the task force committee considered the reagents used during extraction processes, with particular emphasis on samples extracted manually using the ArchivePure Blood Kit (5 Prime Inc.). For the automated DNA extraction protocol all of the reagents are supplied by bioMeriux Clinical Diagnostics and are certified through them. Consequently the automated extraction reagents were not evaluated further by the task force. Reagents used during manual phenol/chloroform extractions likewise were not evaluated by the task force; commercial kits for the manual extraction of specimens other than blood are currently being evaluated by the Laboratory. Validation of these products is ongoing and is likely to result in changes to the reagents used for these extractions in the future. For the ArchivePure Blood Kit (5 Prime Inc), most of the reagents are supplied by the manufacturer; two reagents, ethanol and isopropanol, are not provided in this kit and are purchased separately.
Given DNA degradation in some but not all samples processed using the ArchivePure kit the task force committee first investigated if the ArchivePure manufacturer (5 Prime Inc) or other molecular diagnostic laboratories were aware of any problems pertaining to the general quality or lot-to-lot variations of the kit. To do so the manufacturer was contacted directly. Other Clinical Molecular Laboratories were also surveyed by email to assess how extractions were being performed and if similar inconsistencies in quality of DNA extracts had been observed elsewhere.

To evaluate suitability of the ethanol and isopropanol used in conjugation with the ArchivePure kit, the vendors for each product were contacted directly for information regarding use of these products for molecular genetic techniques (EMD Chemicals Inc., Gibbstown, NJ and Commercial Alcohols Inc, Toronto, ON, respectively).

In addition to the specifications for reagents used in the extraction protocols, the Task Force also considered processes for testing new lot numbers of individual reagents and or kits.

**Evaluation of Acceptance/Rejection Criteria**

*Retrospective examination of test performance for sub-optimal samples*

To better understand if specimen conditions impact the test performance or interpretation, a retrospective study of sub-optimal specimens was conducted. Entries from a six month period of the ArchivePure DNA extraction log book were reviewed to identify samples in which specimen quality issues had been documented by the technician/technologist performing the extraction. A range of sub-optimal specimen conditions were considered including specimens received later than the recommended age (ie. samples that were greater than five days old from the time of collection), specimens that were hemolyzed/clotted and specimens that arrived cold or hot. Using the Molecular Lab database, MLAB, information was subsequently collected for each case regarding the test requested and whether a report was issued (an indication of successful testing). Additional comments in MLAB regarding sample quality were noted when present. If the sample was checked for DNA integrity, the presence of high molecular weight and/or detectable DNA degradation was also noted.

*Critical appraisal of specimen acceptance/rejection criteria*

To address concern raised by the Focus Group that the current criteria are not sufficiently clear or consistently followed the task force committee critically appraised the criteria for acceptance and rejection of specimens sent to CHEO’s laboratory for molecular analysis. Based on evidence from the retrospective studies and experiences processing samples of various sub-optimal conditions, a refined list of criteria was drafted based on various conditions that may be observed at the time of specimen receipt (ie. hemolyzed/clotted blood, tissues received under extreme temperatures, inappropriate test requested, discrepancies between demographics on specimen and requisition, etc.).

*Development of a reporting procedure for rejected and sub-optimal samples*

The Committee recognizes the importance of easy accessibility of the laboratory acceptance criteria and prompt communication of findings pertaining to sample management to the requesting physicians. Towards this goal, the Committee explored methods for educating clients about acceptable specimen criteria and for providing timely notification to healthcare professionals of conditions that may negatively impact the ability of the Laboratory to perform testing. Various resources for educating Laboratory users about specimen requirements were considered including the Laboratory website, information pamphlets, test requisitions and issuance of reports for samples that are either rejected or suboptimal. Input from at least two Clinical Geneticists was sought regarding the utility of rejection/sub-optimal specimen report templates as an education tool.
Evaluation of DNA Storage Conditions

DNA samples stored by the Molecular Genetics Diagnostic Laboratory include samples for immediate genetic testing, residual DNA from genetic tests already performed and samples for long-term storage. Compliance of the Laboratory with recognized practice guidelines for storage of human biological samples was evaluated by reviewing the guidelines published through the International Society for Biological and Environmental Repositories (ISBER) (Pitt et. al., 2008). DNA storage conditions were also compared to reported storage conditions from four other Canadian clinical molecular genetics laboratories. Finally, quality of stored DNA samples was directly measured in a representative group of DNA samples that had been extracted either 6 months or 1 year earlier. DNA samples were identified that had been extracted in either June 3-11, 2011 or January 6-13, 2011. To assess whether DNA quality had been compromised during storage, aliquots of each DNA sample were electrophoresed on an integrity gel and compared to previous gels to assess relative amounts of DNA degradation within the samples.

Evaluation of QA/QC Processes

Assessment of Existing QA/QC Processes

Existing QA/QC processes were reviewed to assess whether they are sufficient for detecting instances of poor DNA quality that may impact downstream testing, or whether further optimization of the process is required. The DNA extraction flow chart and protocols were also examined to identify critical steps of the process where QA/QC measures could be added if available. This was combined with literature searches for genetic quality indicators/metrics that could be used for further development of the laboratory quality assurance program.

Optimization of Integrity Gel Conditions

Although integrity gels have been routinely performed by the CHEO Molecular Genetics Diagnostic Laboratory, specific protocols regarding electrophoresis conditions or the amount of DNA to electrophorese have not been previously established. Inconsistencies in the performance of integrity gels from one gel to the next, coupled with variability in the amount of DNA loaded between wells on the same gel, make comparison of DNA integrity between samples difficult. Standardization of conditions used to perform integrity gels would enhance comparisons between samples/gels and better allow evaluation of suboptimal DNA extracts.

To determine optimal gel conditions for examination of DNA integrity a number of conditions were assessed including DNA concentration, ladder concentration, running voltage and electrophoresis time. Briefly, three different 1% agarose gels were prepared according to the existing Agarose Gel Electrophoresis protocol in Paradigm (approval date 6/21/2010). Each gel contained between 18-19 samples and varied according to the following parameters:

<table>
<thead>
<tr>
<th>Gel</th>
<th>DNA amount (ng)</th>
<th>Ladder amount (ng)</th>
<th>Voltage (Volts)</th>
<th>Electrophoresis Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>500</td>
<td>100</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>250</td>
<td>75</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>50</td>
<td>75</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 1: Integrity Gel Optimization Conditions
Each of the three gels were evaluated for the ability to resolve individual bands and the likelihood for differentiating between sheered DNA and “overloading” of intact DNA.

Development of a protocol for assessing DNA stability in the post-extraction period (3 weeks and 1 year post extraction)

Review of existing Laboratory QA/QC protocols by the Task Force Committee identified a lack of routine processes for the evaluation of DNA quality during storage. Optical density measurements and/or integrity gels are assessed within a week of extraction to determine DNA concentrations, DNA:protein ratios and presence of degraded DNA; however similar measurements are not made after this period unless specifically requested. Inconsistent or infrequent assessment of samples after the initial extraction diminishes the Laboratory’s ability to detect incidents that may negatively impact DNA quality during DNA storage. As such a system was developed to allow routine assessment the quality of stored DNA samples, including proposed schedules and methods for identifying representative samples for analysis.

Evaluation of DNA Send-Out Follow-up Procedures

Independent of the current task force activities, the Eastern Ontario Regional Laboratory Association (EORLA) and its partner hospitals are currently engaged in a cooperative project to build a regional laboratory system (RLIS). As part of this development and to be consistent with several other laboratories in the province, all genetic test results issued by either the Eastern Ontario Regional Genetics Laboratories or outside referral laboratories will be incorporated into the new RLIS system. This includes genetic tests for which DNA is shipped through the Molecular Genetic Laboratory. This feature of the future RLIS will allow better tracking of test send-outs and whether testing has been successful through referral laboratories. Since this change in practice will allow for improved tracking of quality issues involving DNA samples shipped elsewhere, a send-out follow-up procedure was not investigated further by the Task Force Committee. Further recommendations regarding the investigation of any future test failures involving DNA sent elsewhere were developed based upon the investigation performed by the current task force committee activities outlined in other sections of this report.

External Review

An external review of the Task Force Committee activities was solicited to allow for unbiased evaluation of committee activities and identify any perceived gaps that may exist in the investigations performed. A contract was thus signed with Dr. Martin Somerville to evaluate laboratory procedures and committee findings. Martin Somerville is the Director of Medical Genetics Laboratories for the Alberta Laboratory Services and an experienced Molecular Geneticist. Contracted activities are itemized below:

- Review current protocols related to sample receipt, DNA extraction and DNA storage to identify any deficiencies in the protocols that may negatively impact DNA quality.
- Review quality measures collected by the lab over the last year to assess extent of variability in DNA quality over that time period and identify possible trends that may have contributed to reduced DNA quality.
- Propose recommendations for collection of additional DNA quality indicators that are not currently collected, if applicable.
• Review DNA task force meeting minutes (and any associated attachments) and make recommendations for any further investigations that may not have been considered by the task force.
• Provide critical assessment of the final report prepared by the task force prior to its release.

Martin J. Somerville, PhD, FCCMG, FACMG, is a Professor in the Department of Medical Genetics, University of Alberta with adjunct appointments to the Department of Pediatrics, University of Alberta and the Department of Medical Genetics, University of Calgary. He is also the Administrative and Medical/Scientific Director of Genetic Laboratory Services for Alberta Health Services, overseeing eight diagnostic laboratories in Edmonton and Calgary. His recent research activities have focused on clinically relevant genomic copy number variants. This has led to several discoveries of deletions and duplications associated with congenital heart defects, developmental delay, speech defects, and autism. In addition, he has extensive experience in policy development for issues related to quality assurance for genetic testing. He was a member of the Canadian Federal / Provincial / Territorial (F/P/T) Task Force on Genetics and Health, and co-authored the current OECD report on best practice guidelines for clinical molecular genetic testing. He has served as an external reviewer for several initiatives, including the Ontario Predictive Cancer Genetics Program, Repatriation of Genetic testing in Ontario, and is a member of the expert panel on genetic testing for the Ontario Ministry of Health and Long-Term Care. He has also served as a member of the College of Physicians and Surgeons of Alberta Advisory Committee for Laboratory Medicine and as a past President of the Canadian College of Medical Geneticists.
Results and Discussion

Retrospective Investigation of Reported or Observed Suboptimal DNA Extracts

DNA Send-outs: Failed Genetic Tests at Referral Sites

Two reports were obtained in response to a memo requesting copies of any report received from another laboratory indicating insufficient quality of DNA extracted by this laboratory. No additional incidents of test failures were reported.

The two reports of failed genetic testing were both from the same institution (Bioscientia Center for Human Genetics, Ingelheim, Germany) and both were for MLPA analysis of the SHOX gene. The reports indicate that the results were “not analyzable”; the following interpretation was provided in each case:

“The analysis was performed repeatedly without obtaining non-ambiguous signals. Since the test system is optimized for in-house prepared DNA from EDTA-blood, please send for further analyses an EDTA-blood sample of the patient.”

Internal investigation of DNA quality in both cases was performed by electrophoretic examination of aliquots from the remaining sample retained by the Molecular Genetics Laboratory (see Figure 1). There was no evidence of degradation apparent in either sample.

![Figure 1: Integrity gels of remaining samples retained by the Molecular Genetics Laboratory for two cases of reported testing failure at a referral laboratory. Lane 1 includes a DNA ladder. Lanes 2 and 3 include aliquots of the DNA samples discussed above. Observed bands represent high molecular weight DNA. There is no evidence of degradation (i.e., there is no smearing observed of the sample from the high molecular weight band to the lower portion of the gel).](image)

The Task Force Committee further assessed the reports provided by Bioscientia. There was general agreement by the committee that a key factor in the test failure was use of a DNA sample for MLPA studies that had not been extracted at the Bioscientia facilities. Experience with this technique in our own laboratory suggests that the procedure is sensitive to the methodology used for DNA extraction. Because amplification of test samples is compared to amplification of control sample in determining gene dosage, unambiguous copy number assessment can be influenced by factors that differentially impact amplification of either the test or the control. Although MLPA studies are possible using DNA extracted by different methods, repeat analysis to obtain non-ambiguous results is considerably more common using DNA extracted by methods different than controls than with samples extracted by the same methodology. Successful sequencing by Bioscientia of the SHOX gene using the same aliquots as submitted for MLPA studies is consistent with the hypothesis that the failure is related to the type of extraction used rather than the quality of the DNA.
**Recommendations:**

- Health care providers should continue to be encouraged to send peripheral blood samples to referral laboratories whenever possible. This practice provides the referral laboratory with back-up samples should it be required and does not compromise testing optimized for specific DNA extraction protocols.

**Sporadic Incidence of Degraded DNA within the CHEO Molecular Genetics Diagnostic Laboratory**

Retrospective analysis of integrity gels performed between January and June 2011 allowed the identification of 88 samples with degraded DNA. The amount of degradation was classified as “minor”, “moderate” or “major” depending on the amount of sheared DNA visible on the gel, and the presence of high molecular weight in the sample. Examples of degradation classified as “major” and “moderate” are shown in Figure 2a; this is compared to a recent integrity with no evidence of degradation (refer to Figure 2b). Of the 88 samples identified, 37 samples were excluded from further investigation of duplicate samples or because it was not possible to assess some of the parameters for study.

Of the remaining 51 samples studied further, there were no trends identified that could consistently explain how samples became degraded (refer to Appendix A). Most of the degraded DNA samples investigated were extracted from peripheral blood specimens received from CHEO; it was felt that this reflects a high proportion of total laboratory specimens coming from CHEO rather than there being an issue with shipment of specimens from the CHEO blood draw laboratory/inpatient units to the Molecular Genetics Laboratory. Degradation of samples was not limited to a specific technologist or technician performing the extraction; all of the technologists/technicians rotating through the DNA extraction bench handled samples which showed evidence of degradation. There was no correlation observed between the age of samples and the amount of degradation observed. Finally, while some batches of extractions were identified in which all of the samples were degraded, there were also batches of extractions that included both samples with intact DNA and samples with degraded DNA. Instances with degradation of all of the samples in a single batch suggest a possible issue with the extraction process or reagent used, whereby the observation of successful and suboptimal extractions in the same batch suggests that the problem was specific to only a subset of the samples.

![Figure 2: Sporadic Incidence of DNA degradation in period prior to Task Force.](image)

**DNA Extraction Process – Overview and External Assessment**

The pre-analytical, analytical and post-analytical aspects of the DNA extraction processes were all examined as part of the walk through with the Laboratory Quality Coordinator and Senior Technologist from Virology. These processes are summarized in a flow chart that depicts the flow of specimens from sample receipt to sample
storage (refer to Appendix B). The processes for sample submission and accessioning are the same despite the type of extraction method used. After accessioning samples are separated into racks based on which protocol will be used for the extraction, i.e. automated extraction on the bioMerieux EasyMag instrument, embedment of cells in an agarose plug, or manual extraction using either the ArchivePure Blood kit or a phenol/chloroform extraction protocol. Because downstream testing of samples extracted using the EasyMag instrument does not require high molecular weight DNA, and because agarose plugs and phenol/chloroform extractions are infrequently used, most of the attention for the walk through and subsequent Task Force Investigations were focused on the extractions using the ArchivePure Blood kit.

A number of discrepancies were noted between the extraction processes performed in the Molecular Genetics Laboratory compared to those performed in the Virology Laboratory. Many of these discrepancies were related to difference in requirements for preservation of viral nucleic acids in Virology versus preservation of high molecular weight DNA in Molecular Genetics. Despite these differences, three key discrepancies were identified that had the potential to impact quality of either individual samples or of the entire batch. Firstly, it was noted that DNA extraction reagents were stored on a cart in front of a window. As reagents may be susceptible to temperature fluctuations in this location, it was recommended that they should be moved to a more stable location outside of direct sunlight. This has already occurred. Secondly, “back-up” blood specimens had been stored at room temperature until the success of the extraction could be confirmed. Consequently samples may be held as long as 14 days at room temperature prior to use as a back-up. The Laboratory routinely prepares an extra buffy coat pellet for samples with sufficient volumes of blood. These buffy coats are stored at -20°C and are used for preparation of back-up samples when ever possible. Since the walk through, when extra specimen tubes are available that aren’t used immediately for DNA extraction or preparation of a frozen buffy coat, the extra tubes are stored for one week at 4°C rather than at room temperature then disposed. After the one week period, if an extraction fails and no buffy coat is available, then the referring physician is contacted to request a repeat specimen. Lastly, it was noted that ultraviolet light was not being used to decontaminate the surfaces where DNA is extracted. The fume hood and plastic consumable products used in the extraction process should be treated with ultra violet radiation daily to minimize risks of DNA contamination from these surfaces.

Recommendations:
- ArchivePure kit reagents should be stored in a location free from extreme temperature fluctuations.
- Extra specimens received for an individual patient should continue to be stored as a frozen buffy coat for potential use as a “back up” sample. Any additional extra tubes can be stored for up to one week at 4°C. After this period, if the frozen buffy is deemed unacceptable or repeat extractions provides insufficient DNA, then a repeat specimen should be requested.
- Fume hood and plastic consumable products used in the extraction process should be treated with ultra violet radiation daily to minimize risks of DNA contamination from these surfaces.

DNA Quality Focus Group

The focus group successfully met on May 26, 2011. An invitation to participate in the focus group was extended to all of the Genetics PSU technologists who were certified in Molecular Genetics, to all of the committee members, to the Laboratory Scientists and to the senior technologist from the Virology Laboratory. There was enthusiastic participation of thirteen individuals including one medical laboratory assistant, two scientists, and ten technologists.
from three different clinical laboratories at CHEO (Molecular Genetics Diagnostic, Cytogenetics, and Virology Laboratories).

Comments related to each of the topics were received from all of the individuals attending the focus group. There was considerable discussion related to the first three topics: acceptance/rejection criteria, DNA quality measurement, and DNA extraction protocols, however discussions were more limited for the latter topics due to time restrictions. All of the participant’s comments are summarized in Appendices C and D.

Regarding sample acceptance and rejection criteria there was a general consensus that clarification and strict adherence to acceptance/rejection criteria was required. This was an item of obvious concern for the participants and was commented on in the notes of 11/13 individuals. At the time of the focus group meeting an approved protocol existed with sample acceptance/rejection criteria but it was generally felt that the criteria were somewhat poorly defined and inconsistently followed. Another theme that was raised by a number of individuals in the group was communication between the Molecular Genetics Diagnostic Lab and blood draw facilities/ laboratories/ health care professionals referring samples. Three individuals recommended education to external sites regarding sample conditions, possibly through development of an information pamphlet. Two other individuals also put forth suggestions for a reporting system to inform providers of unacceptable or sub-optimal specimen conditions.

When asked about DNA quality measurements, most of the participants felt that the integrity gels (7/13 individuals) and optical density readings (6/13 individuals) were of value and should be continued. Related to this, there were also suggestions that factors influencing reproducibility of these two measurements should be examined (ie. homogeneity of reconstituted DNA, consistency in electrophoretic conditions). In addition to the existing direct DNA measurement several individuals (6/13) suggested that the specimen conditions upon receipt should be clearly recorded to provide information during downstream testing about samples that may have been compromised, including information about insufficient specimen volume, temperature extremes, and excessive time from procurement.

Considerable variety is noted regarding focus group comments about the DNA extraction protocol. Five of the thirteen participants indicated that consistency in the extraction protocol should be established. Similarly, three individuals suggested that the laboratory protocol should be altered to restrict deviations from the manufacturer’s recommended protocol. There were also a number of comments related to limitations in batch sizes, use of small reagent aliquots, and minimizing distractions or delays during the extraction process.

Despite limited time for discussion of the remaining three topics, participants were able to provide written comments which have also been collated in Appendices C and D.

Focus group comments were discussed in detail by the Task Force Committee and prioritized as items that were amenable to investigation by the committee, items that could be recommended by the committee without further investigation and those that were beyond the committee resources or scope and would not be followed up further. Further follow-up is summarized in each of the relevant sections below.

**Literature Search**

A literature search for articles related to factors that influence DNA quality (ie. number of days from specimen collection, hemolysis, blood clotting, rehydration conditions and residual alcohol in reconstituted samples) was performed by the Library manager, however no suitable journal articles were identified by this search.
Protocol Refinement

Prior to January 2011, much of the work in sample accessioning and DNA extraction was performed by a single medical laboratory assistant for the approximately 4,500 specimens received annually. From January to May, 2011, DNA extractions were also performed by technologists; individuals were scheduled to perform extractions one day per week. The rotation schedule allowed for DNA extractions to proceed while permitting technologists to continue performing genetic tests assigned to them. On May 4 a second medical laboratory assistant was hired for a one year term position. To accommodate training of the second technician, the rotation of technologists through the DNA extraction bench persisted until the new staff member was adequately trained to allow transition of the responsibility of DNA extraction procedures back to the medical laboratory assistants.

Concern was raised by a number of individuals in the Focus Group about whether inconsistencies in the performance of DNA extractions were a reflection of the fact that multiple individuals performed the extractions on an irregular basis; individuals, although following the protocol, perform the extractions differently. To this extent, the technologists and technician performing the extractions were shadowed to evaluate if there were minor differences in technique that may impact DNA quality. The existing ArchivePure extraction protocol was also reviewed in consultation with the manufacturer to ensure that it was consistent with the manufacturer-recommended procedures.

A number of deviations from the protocol were observed and have been summarized by an independent reviewer of the shadowing notes. Some of these deviations were minor such as use of a pipette to mix samples rather than a vortex; however some of the deviations were substantial, including omission of a step in one instance. It has been suggested that ambiguity of the protocol and procedural requirements may have contributed to these discrepancies. A further suggestion had been made that perceived pressures related to other bench responsibilities may have impacted the technologist’s ability to focus on the task of DNA extraction.

Discussions with the ArchivePure Blood kit vendor highlighted the existence of critical steps that could lead to DNA degradation if the protocol is not followed according to the recommended procedures, i.e. vortexing samples for too long. The current Molecular Genetics Laboratory protocol for use of this kit (approval date: May 11, 2011) was evaluated with consideration of the manufacturer’s suggestions for kit performance; the written protocol was found to be in accordance with the suggested procedures outlined by the vendor. Despite this, there were some written steps that were not explicit, thus making them susceptible to variable interpretation by technicians and technologists performing the extraction.

The technologists covering the DNA extraction bench from January to May are to be commended on their commitment to service maintenance and teamwork to make the rotations successful. It is recognized that while unforeseeable events necessitated coverage of the DNA extraction bench during this period, the rotations were not an ideal situation for ensuring consistency in assay performance on this bench. Analysis of the shadowing notes and results from retrospective investigations of DNA clearly showed that the sporadic incidents of degradation were unrelated to a specific technologist. In light of the inconsistencies observed between technologists and the perceived vagueness of the protocol the protocol was revised to ensure clarity, eliminate ambiguity in performing certain steps, and provide guidance for extraction of problematic samples. The revised protocol is still in draft format, but should become approved in a timely fashion to minimize inconsistencies in extraction performance. A back-up plan is also required to provide more consistent coverage of DNA extraction duties should such coverage be required in the future. Employment of a second Medical Laboratory Assistant has allowed improvements in this regard, however since this is a term-position the second technician would not be available to provide coverage once the period of the term has been completed.
**Recommendations:**

- The approved protocol for extraction of DNA using the ArchivePure kit (“DNA Extraction – ArchivePure DNA Blood Base Kit for Fresh or Frozen Blood or Buffy”, approval date: May 5, 2011) has been re-examined with consideration of the procedural discrepancies observed above and the discussions with the vendor regarding critical steps. A draft of the revised protocol will be completed and approved to ensure directions and assay performance requirements are sufficiently clear to minimize inconsistencies in assay performance between users.

- A plan for providing coverage on the DNA extraction bench should be developed that does not rely on daily rotations of technologists. Any developed plan should allow protected time for the person providing coverage to perform the duties of the DNA extraction bench without also managing other laboratory activities.

- Given the large volume of samples that are processed by the Laboratory each year, a minimum of two individuals should be available to cover duties in the DNA extraction and specimen receiving rooms at all times.

- Establishment of cut-offs for time of specimen receipt and batch sizes may be considered by the laboratory to aid in appropriate batching of DNA extractions.

**Reagent Evaluation**

For manual DNA extractions using the ArchivePure kit, all of the reagents are supplied by the manufacturer with the exception of ethanol and 70% isopropanol. The kit reagents are certified by the manufacturer and come in sufficient volumes to allow extraction of ~330 specimens per kit. To avoid contamination of large volumes of reagents and ensure that they are fresh for each batch of extractions, smaller aliquots of the kit reagent are prepared. A number of recommendations related to kit reagents were suggested by the focus group and are supported by the Task Force Committee. Specifically, it is recommended that reagent aliquots should be disposed rather than refilled when there are insufficient volumes remaining. It is also recommended that aliquots should be discarded within two days of being opened to ensure that they are fresh. Finally, inconsistencies in the DNA reconstitution step of the protocol were noted by the focus group. It is recommended that wide-bore tips be used during this step as these are better designed for assessment of sample viscosity than standard tips.

With regards to ethanol and 70% isopropanol, the suppliers for both reagents were contacted directly to enquire about their suitability for DNA extraction protocols (EMD Chemicals Inc. and Commercial Alcohols Inc., respectively). Neither reagent complied with the molecular grade requirements recommended for high quality DNA extractions. The committee has subsequently identified at least one source for high molecular grade ethanol and isopropanol (Fisher Catalog # BP2818-4, or BP2818-500 and Fisher Catalog # BP26818-4, BP2618-212 or BP2618-1, respectively). Prior to adopting changes to the extraction procedure using the molecular grade alcohols, it is recommended that they should be appropriately validated for use in the protocol. Towards this goal, a minimum of 5-10 peripheral blood samples should be examined and extracted in parallel using the old and new reagents. Optical density measurements and integrity gel analysis are recommended to ensure purity and integrity of the duplicated DNA samples.

A final item regarding reagents that was considered by the Task Force Committee included evaluation of new reagent/kit lot numbers. New kits and reagents used for disease-specific testing protocols are tested when introduced by virtue of including previously tested controls in each test batch. However, similar controls are not currently used to test new lots of reagents for DNA extraction. To meet this goal a process was discussed
whereby samples are extracted in duplicate using old and new reagents. Duplicates of at least two samples should be used to test the new reagent lot number in case one of the samples has unknowingly been compromised. All duplicate extractions are to be electrophoresed on an integrity gel to ensure similar results prior to the introduction of the new lot number into service. A logbook of all new lot number tests should be maintained. It is also recommended that as much as possible bulk purchases of the same lot number should be made to minimize the amount of new lot number testing required.

**Recommendations:**

- Reagent aliquots should be disposed when insufficient volumes are left, rather than being “topped-up” with fresh reagents.
- Reagent aliquots should be disposed within two days after being opened, regardless of whether they are finished.
- Use of wide-bore tips should be incorporated into the final DNA reconstitution steps to better assist in assessing sample viscosity compared to standard tips.
- The Laboratory should identify a supplier for molecular grade ethanol and isopropanol and validate their use with the ArchivePure protocol prior to implementation.
- New lot numbers of reagent or kits should be validated prior to being introduced to service using either the process developed above or an equivalent one.

**Evaluation of Acceptance/Rejection Criteria**

**Retrospective examination of test performance for sub-optimal samples**

Retrospective investigations of sub-optimal specimens were performed i) to determine if any of the suboptimal specimen conditions frequently observed in the Laboratory show correlations with poor DNA extraction results, and ii) to determine the impact of suboptimal specimen conditions on testing and obtaining test results. A summary of the retrospective examination of test performance for sub-optimal samples is shown in Appendix E.

Thirty blood samples were identified where the technician/technologist documented issues with the sample prior to or during the extraction process. One sample was rejected and not processed. Of the 29 processed samples, 20 were checked on an integrity gel with the majority of samples showing signs of DNA degradation. Two samples yielded insufficient DNA quantity to produce a test result. The remainder of samples generated sufficient quality test results to permit analysis and interpretation of findings for a report. However, some of these cases had normal test findings by PCR and may have required a repeat sample for continuation of testing if Southern Blot analysis had been required. The results suggest that while the majority of these samples displayed visual signs of compromise it was still possible to interpret findings for many of them; it was not possible to predict which samples generate poor quality DNA based on these visual signs of compromise alone.

It has been suggested within Task Force Committee discussions that a similar audit to the one above may be useful as a quality assurance program within the laboratory. Performance of a similar survey is therefore recommended on an annual or semi-annual basis.

**Recommendations:**

- Retrospective analysis of test performance for sub-optimal samples should be performed on either an annual or semi-annual basis.
Critical appraisal of specimen acceptance/rejection criteria

Particular concern was raised by the focus group regarding the current acceptance/rejection criteria within the Laboratory. Although a protocol has been approved, many of the focus group participants commented that the criteria were poorly defined and inconsistently followed. As such, the approved criteria were reviewed and changes to the protocol have been made to better define when samples should be accepted or rejected according to the following rationales. The revised protocol remains in a draft format and would not apply to “irretrievable samples”; wide dissemination of the revised rejection criteria must occur prior to the adoption of these modified criteria.

The Task Force Committee agreed that the following core conditions should be met before sample processing is initiated:

- The requested test (or DNA banking) should be performed by the CHEO Molecular Genetics Diagnostics Laboratory;
- Peripheral blood specimens must be received in a EDTA or ACD tubes;
- Demographics on the specimen should match with those on the requisition;
- If immediate sample send-out to a referral laboratory is requested, all of the appropriate accompanying documentation must be submitted (including referral laboratory requisitions and any required Ministry of Health out-of-province testing approval; if out-of-province testing is pending the sample can be banked until appropriate approval is received).

If any of these core conditions are not met, the Committee recommends that the sample should be rejected and sample processing should not be initiated.

It has been agreed that samples should generally be rejected if received after 3 days for FSHD or myotonic dystrophy type 2 testing and 7 days for all other tests (collection date = day 1), as the quality of DNA in these samples is often compromised. However, the committee recognized that exceptions for “irretrievable” or out-of-province samples may be necessary. Therefore, the committee recommends that samples received after the acceptable transit time should be brought to the attention of the Molecular Geneticist on service.

The Committee recognized that other observations, such as hemolysis, clots and extreme temperatures, can indicate that the sample has been compromised, however, it is unclear how often these factors negatively impact testing. While hemolyzed, cold or hot samples often do not require any alternative treatment, processing of clotted blood samples is labor intensive and requires intensive manual intervention (e.g. vortexing and physical crushing of the blood clots). For these reasons, hemolyzed, cold or hot peripheral blood specimens should be processed given that the other acceptance criteria are met. Peripheral blood specimens that are received with obvious clots should be immediately rejected.

The issue of accepting samples on Fridays or before long weekends was also discussed. To ensure appropriate processing of samples in a timely fashion, the Committee recommended establishing a strict deadline for shipment, i.e. samples must be received by a set time on the day before the weekend.

**Recommendations:**

- Based on the focus group suggestions and task force discussions, it is recommended that clearer acceptance and rejection criteria be adopted and adhered to. Revised acceptance/rejection criteria should be made available to Laboratory users prior to implementation.
A system should be developed to ensure consistent recording of sub-optimal specimen or extraction conditions that allows easy access to this information during all downstream analytical and post-analytical processes.

Development of a reporting procedure for rejected and sub-optimal samples

The Committee recognizes the importance of easy accessibility of the laboratory acceptance criteria and prompt communication of findings pertaining to specimen management to the requesting physicians. Towards this goal, the Committee recommends that the specimen acceptance criteria should be listed on the CHEO website, in the ward manual, and on the requisition. It has also developed report templates for the immediate reporting of samples that do not meet the acceptance criteria so that another sample can be drawn if required (refer to Appendix F). A preliminary reporting tool was also developed for specimens that satisfy the acceptance criteria but are received under “suboptimal conditions” (refer to Appendix G). The “suboptimal report” will indicate the condition observed and inform the requesting physician that testing will proceed but may be compromised. If the testing of the suboptimal sample received is unanalyzable, a new sample would be requested.

The “rejection” and “sub-optimal” reports were developed with checkbox lists of unacceptable/unsatisfactory sample conditions to facilitate further education of laboratory acceptance criteria should another sample be sent to the lab in the future. The utility of these reports and the checkbox lists were discussed with three different Clinical Geneticists; these Geneticists were generally supportive of the concept.

Recommendations:

- Refined acceptance and rejection criteria should be easily accessible to health care professionals using the Laboratory’s services. Specimen requirements should be made clear on the requisition, in the ward manuals, and on the Laboratory website page, including requirements for acceptable transportation times, collection tubes, and blood volumes. Any other specimen conditions that may result in rejection of the specimen should also be clearly indicated in the same locations, i.e. clotted blood.
- Specimen conditions that may compromise DNA quality and test performance should be clearly indicated on the website and in the ward manual, including (but not limited to) extreme temperatures of specimens, hemolysis, and samples that are older than 3 days for FSHD and myotonic dystrophy, type 2 testing, or older than 5 days for other tests.
- A report should be generated for all rejected or “sub-optimal” specimens that clearly indicates the condition observed and whether an additional sample is required.

Evaluation of DNA Storage Conditions

Best Practice Guidelines for the storage of human biological material were published by the International Society for Biological and Environmental Repositories (ISBER) in 2008. This document is primarily targeted to repositories that store samples for research purposes. Much of the material was therefore not relevant to storage of clinical DNA samples, however several specific recommendations were listed that could impact quality of stored DNA. The Task Force Committee determined that the recommended guidelines for the monitoring and maintenance of storage units are currently being followed by staff in the Molecular Genetics Diagnostic Laboratory. In accordance with OLA standards, daily records of fridge/freezer temperatures are kept and reviewed monthly by the senior technologist in an effort to identify trends that may indicate decreased...
performance levels of storage equipment. Furthermore, qualified personnel from the physical plant department at CHEO perform regular preventative maintenance to ensure proper functioning of units. Additional recommendations for emergency power and alarms on units are already in place. In the event of equipment failure, back-up storage capacity for specimens has been deemed adequate. Labels and ink on storage containers were intact and legible in a recent inventory of banked samples. The ISBER document also highlights a requirement for communication of errors between sending and receiving sites for shipped specimens. This requirement is addressed in the section of this report entitled “Evaluation of DNA Send-Out Follow Up Procedures”. Finally, the ISBER best practice guidelines recommended an inventory system of stored specimens. This would be useful in keeping track of the location of samples as well as being able to monitor available storage space. Although a detailed log of individual sample locations is not currently maintained, this will be achievable with the introduction of the new R-LIS program scheduled for 2012.

In addition to reviewing the above guidelines, other laboratories were also informally surveyed regarding their DNA storage conditions. One of the four labs who replied to the survey questions stored their DNA at -20°C. The remaining three labs indicated that they store their DNA at 4°C, consistent with the practices in this Laboratory. It was therefore felt that storage at a different temperature was not warranted.

**Recommendations:**

- To improve tracking of individual banked DNA samples a log of banked samples and their locations should be developed. This potentially could be incorporated into the RLIS system under developed or future LIS versions, depending on scope and budgets for these systems.

Beyond policy review related to storage of DNA samples, the Task Force Committee also directly measured DNA stability of samples stored in the laboratory (refer to Appendix H and I). A subset of DNA samples extracted 6 months and 1 year previously were retrieved. To determine if there were any changes in DNA integrity over the period of storage, 6 month and 1 year Integrity gels were further compared to gel images from the post-extraction period for each sample. No appreciable differences in DNA integrity between the two time points was observed. Optical density readings were also repeated and were similar between the two time points for most samples, suggesting that the DNA solution was homogenous and well reconstituted. Four of 35 samples showed >10% difference in the concentrations calculated between the two optical density readings, indicating that either one of the readings was inaccurate or the sample was not well reconstituted. Attempts to better dissolve the DNA solution should be done for similar cases of discrepant optical density measurements in the future.

Evaluation of QA/QC Processes

Laboratory quality assurance programs were evaluated by the Laboratory Quality Manager and indirectly discussed through the focus group meeting. There was a general agreement by the focus group participants that integrity gels and optical density readings add value to the quality assurance program and should be continued. In addition, the Laboratory Quality Manager identified a number of quality indicators in the literature (Harper *et. al.*, 2010; Berwhouts *et. al.*, 2008; Patton *et. al.*, 2006; Howaritz, 1991). A subset of these are currently assessed by the Laboratory. The list of quality indicators was reviewed and suggestions for their incorporation are outlined in Appendix J.

**Recommendations:**

- Refer to Appendix J.
Optimization of Integrity Gel Conditions

Retrospective analysis of integrity gels highlighted inconsistencies in the parameters used for electrophoresis between gels. High concentrations of DNA were also frequently loaded making inter- and intra-gel comparisons difficult. To aid comparisons between gels and identify appropriate electrophoresis conditions for the easy identification of poor DNA sample conditions, a number of different gel conditions were assayed (refer to Appendix K). Ultimately 50 ng of DNA, 50 ng of ladder, 75 Volts of current and a electrophoresis time of 60 minutes was chosen as the ideal integrity condition for assessment of DNA quality and comparison of samples.

**Recommendations:**

- To allow for inter- and intra-gel comparisons, integrity gel protocols should be revised to include electrophoresis requirements that would better ensure consistent performance of integrity gels. Revised protocols should include specifications for the voltage and time requirements for electrophoresis of integrity gels and amounts of DNA to be loaded for each sample on the gel.
  - Recommended electrophoresis conditions include loading 50 ng of DNA (for samples and ladders), and electrophoresing the samples for 60 minutes at 75 Volts on a 1% agarose gel.

Development of a protocol for assessing DNA quality in the post-extraction period (3 weeks and 1 year post extraction)

In review of Laboratory processes, a lack of quality assurance programs was noted related to measurement of DNA quality over time. The Task Force Committee considered assessment of stored DNA at various time points and ultimately agreed that quality measurements were important shortly after the initial extraction and at least once during long term storage. Specifically, the committee agreed that three week and one year time points would be the most appropriate for quality assessment in this Laboratory. DNA evaluation at the three week time interval allows rapid identification of any DNA degradation that may occur as the result of DNase or other substances that may be in the extract. Although such cases were not directly observed, the committee generally agreed that if a sample was to become degraded because of something in the solution, the degradation would likely occur within the first few weeks post-extraction. The one year assessment further allows evaluation of DNA storage conditions and measurement of stability over an extended period of time.

One of the items most discussed in relation to the three week and one year quality assessments was how to determine which samples should be examined. A system is proposed whereby all banked samples are examined on a integrity gel at the time of extraction and again three weeks later (refer to Appendix L). To further identify samples for examination at the one year interval, DNA pulled from the 4°C fridge for “send-out” to an external laboratory will be put aside in one of twelve boxes based on the month of extraction and then returned to the 4°C fridge. Samples collected in this fashion will subsequently be evaluated on an integrity gel one year post extraction. This allows examination of a new batch of representative one year old samples each month (refer to Appendix L).

Finally, to assess whether samples are well reconstituted in solution optical density measurement will be repeated at the same time that 3 week and 1 year integrity gels are performed. If the sample is dissolved uniformly within solution then calculated DNA concentrations (based on A260/A280 optical density readings) should be similar between the original measurements and the one at either 3 week or 1 year post extraction. Large differences between the measurements in each time period suggests that the sample is heterogenic and that sample reconstitution should be repeated.
**Recommendations:**

• Stability of stored DNA should be assessed monthly by performing integrity gels of samples extracted 3 weeks and 1 year previously. (A proposed process for 3 week- and 1 year-gels is described above).

• Optical density readings should be performed for all samples examined on the 3-week and 1-year integrity gels and compared to original readings to allow monitoring of consistency of DNA reconstitution. Variability in optical densities between the time periods could suggest issue related to DNA reconstitution and additional processing of samples may be required.

**Evaluation of DNA Send-Out Follow-up Procedures**

The development of a regional LIS program through the EORLA organization is timely in that it will allow the Molecular Genetics Laboratory a mechanism for tracking results from genetic tests sent to referral laboratories. Directly receiving copies of these reports and having a system to track outstanding results would permit the Molecular Genetics Laboratory to promptly identify possible DNA quality issues involving DNA sent elsewhere, and conduct appropriate investigations in a timely fashion. The practice of having the Laboratory copied on all reports for samples sent out further minimizes the risk that the Laboratory is not informed of DNA quality concerns raised by referral labs should they occur.

Based on the experiences of the Task Force activities, this committee recommends that investigations of any potential DNA quality issues should include at a minimum, evaluation of the report (and possible contact with the referral laboratory) to assess the nature of the potential quality issue, and when sufficient DNA is available repeat electrophoresis/absorbance readings for each investigated samples. Pedigree charts, RLIS/MLAB, and the chart communication may also be examined for notes that may indicate that the sample had been compromised. Other specimens extracted in the same batch may also be investigated to assess whether the quality issue raised is isolated or whether the whole batch is affected. A repeat specimen should be requested if the sample is found to be of poor quality.

**Recommendations:**

• The Laboratory should be copied to all reports for DNA samples sent to external laboratories to allow tracking of completed tests through referral centers and initiate appropriate investigations if quality issues regarding sent DNA are raised. The Task Force accepts that implementation of this recommendation would be dependent on the availability of sufficient resources prior to the implementation of the new RLIS (Cerner Millenium).

• To investigate any issues related to quality of DNA sent to referral labs, an aliquot from the sample should be electrophoresed on an integrity gel to assess if there is any degradation. Optical density readings should also be repeated to ensure that there are adequate amounts of DNA and that measurements are consistent with previous measurements. Additional investigations may include, but are not limited to, communication with the referral laboratory and investigation of other samples extracted in the same batch.
External Review

A report from the external reviewer regarding his assessment of the Task Force Committee activities was not available for inclusion in this report at the time of its submission. Dr. Martin Somerville’s review will therefore be submitted as an appendix to the current report when it becomes available.

Revision statement (September 13, 2011):
An external review of Laboratory protocols, integrity gels, task force committee meeting minutes, and other documents has been completed by Dr. Martin Somerville. His findings and recommendations related to this review are included “verbatim” in Appendix M.
References


Howanitz PC. (1991). Q-Probes: a tool for enhancing your lab’s QA. Medical Laboratory Observer.


Appendix A – Specimen Processing Flowchart: From receipt to storage

Workflow for DNA Extraction

Samples received in laboratory

- Sorting Receiving Re-routing
  - Samples at RT
  - STATS or expedited put to front

- Separate EasyMag and Archive Pure
  - Check history Add pedigree

- Enter data into LabDemog

- Quick Labels

- File Making

- Label Samples (add comments)

- Separate and rack

  Dedicated pipets, gloves all reagents, water purchased.
  NOTE: Mol ID 2900 rpm 10 mins, do not use buffy coat

FSH plugs

 Archive pure (buffy coat)

- Spin (15 mins @ 2000 rpm)

- Rin buffy coat, add RBC lysis

- Spin, rinse buffy coat, add RBC lysis

- Spin, work with supernatant

- Add 3ml of 100% isopropanol with 2
  washes of 70% ethanol

- Heat to evaporate

EasyMag Extractor (whole blood)

- EasyMag trays
  (1524 per run)

- Add DNA Hydration solution, leave overnight at RT

- Heat to dissolve (in morning)

- Check OD and use integrity gel as applicable
Appendix B: Retrospective Investigation of Degraded DNA Samples

Refer to attached Excel Worksheet entitled “Appendix B”.

Appendix C: Itemization of Focus Group Comments

Refer to attached Excel Worksheet entitled “Appendix C”
Appendix D: Fishbone Diagram to Summarize Focus Group Comments

- QA/QC Processes
  - Request for info on all send outs
  - Simplify process for send outs
  - Education for providers
  - Ship whole blood when possible
  - Report from all physicians, counselors

- DNA Storage
  - Temp monitoring
  - Timings at different temps
  - Storage and aliquot of alcohols
  - Discard protocols
  - DNA bank
  - Lyophilize DNA
  - Integrity gels run over time for stored DNA

- Extraction Supplies and Reagents
  - Record lot #
  - Storage and aliquot of alcohol
  - Discard protocols
  - DNA bank
  - Lyophilize DNA
  - Integrity gels run over time for stored DNA

- DNA Extraction Protocol
  - Smaller aliquots of DNA solutions
  - Review critical steps in protocol
  - Consistency in process and extraction
  - Hydration, DNA dissolution, lysis
  - Extraction in 1 day, no delays or multi-tasking
  - No deviations from manufacturer's guidelines
  - Test new lot # prior to use
  - Establish cut off times and sizes for batches
  - Sample storage between steps

- Quality DNA Generation
  - Establish clear acceptance/rejection criteria
  - Education to external locations
  - Reporting system for rejected samples and follow up
  - Define irretrievable samples
  - Define acceptance/rejection criteria
  - Report for non-ideal samples
  - Procedure for Fridays and long weekends
  - Records of communication between physician offices
  - Tracking of compromised samples
  - Quick labels for processing

- Sample Acceptance/Rejection Criteria
  - Mark aliquot if poor sample
  - Storage of A.P. Kite

- DNA Quality Indicators
  - Record sample conditions on receipt
  - Record OD readings in MLAB
  - Maintain OD 260/280 readings
  - Maintain use of integrity gels
  - Correlation of successful testing
  - Factors affecting reproducibility
  - Examine dissolved DNA
  - Record receipt, extraction method
  - Prioritize sample processing
  - Record sample split or multiple
  - Records of communication between physician offices

- Notification of Testing Failures at External Sites using CHEO DNA
  - Unsuccessful testing
  - Reporting of failures to external sites
  - Communication with external sites
## Appendix E - Retrospective examination of test performance for sub-optimal samples

<table>
<thead>
<tr>
<th>Date of Receipt</th>
<th>Lab No</th>
<th>Comment</th>
<th>Gel Findings</th>
<th>Disease</th>
<th>Reportable Result Achieved</th>
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<tr>
<td>26-Jan</td>
<td>56034BD</td>
<td>clotted; manually removed clot after RBC lysis</td>
<td></td>
<td>DM</td>
<td>yes</td>
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<tr>
<td>4-Feb</td>
<td>56170BD</td>
<td>hemolyzed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-Feb</td>
<td>56194BD</td>
<td>clotted; o/n @ 50 with prot K</td>
<td>HMW, ++</td>
<td>MD2</td>
<td>yes</td>
</tr>
<tr>
<td>14-Feb</td>
<td>56275BD</td>
<td>tubes felt cool</td>
<td>MD2</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>17-Mar</td>
<td>56727BD</td>
<td>a bit blood red</td>
<td>HMW</td>
<td>Bank</td>
<td></td>
</tr>
<tr>
<td>17-Mar</td>
<td>56720BD</td>
<td>a bit blood red</td>
<td>BC</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>17-Mar</td>
<td>56728BD</td>
<td>a bit blood red</td>
<td>CF</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>17-Mar</td>
<td>56722BD</td>
<td>a bit blood red</td>
<td>DM</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>17-Mar</td>
<td>56726BD</td>
<td>a bit blood red</td>
<td>DM</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>23-Mar</td>
<td>56797BD</td>
<td>blood very dark/black</td>
<td>HMW</td>
<td>Bank</td>
<td></td>
</tr>
<tr>
<td>23-Mar</td>
<td>56795BD</td>
<td>blood very dark/black</td>
<td>BC</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>23-Mar</td>
<td>56772BD</td>
<td>sample &gt; 6 days; looks hemolyzed</td>
<td>HMW, +</td>
<td>FRAX</td>
<td>not needed</td>
</tr>
<tr>
<td>23-Mar</td>
<td>56773BD</td>
<td>sample 6 days old</td>
<td>no DNA</td>
<td>FRAX</td>
<td>ND</td>
</tr>
<tr>
<td>30-Mar</td>
<td>56903BD</td>
<td>6 day old sample, very hemolyzed</td>
<td></td>
<td>FRAX</td>
<td>yes</td>
</tr>
<tr>
<td>30-Mar</td>
<td>56911BD</td>
<td>sample very very cold, very hemolyzed; only 3ml</td>
<td></td>
<td>MD2</td>
<td>ND; result obtained using another sample from the same patient</td>
</tr>
<tr>
<td>1-Apr</td>
<td>56948BD</td>
<td>clotted; 1 big clot</td>
<td>HMW</td>
<td>Bank</td>
<td></td>
</tr>
<tr>
<td>15-Apr</td>
<td>57209BD</td>
<td>hemolyzed</td>
<td>no DNA</td>
<td>FRAX</td>
<td>no - PCR failed</td>
</tr>
<tr>
<td>20-Apr</td>
<td>57262BD</td>
<td>clotted: took everything but the clot</td>
<td>HMW</td>
<td>Bank/Send</td>
<td>? (no failed report rec'd)</td>
</tr>
<tr>
<td>20-Apr</td>
<td>57253BD</td>
<td>&gt; 5 days old; hemolyzed</td>
<td>HMW, ++</td>
<td>MD2</td>
<td>yes</td>
</tr>
<tr>
<td>26-Apr</td>
<td>57298BD</td>
<td>&gt; 5 days old</td>
<td>HMW, ++</td>
<td>Bank</td>
<td></td>
</tr>
<tr>
<td>26-Apr</td>
<td>57318BD</td>
<td>5 days old</td>
<td>HMW, ++</td>
<td>Bank</td>
<td></td>
</tr>
<tr>
<td>26-Apr</td>
<td>57323BD</td>
<td>5 days old</td>
<td>HMW, ++</td>
<td>BC</td>
<td>yes</td>
</tr>
<tr>
<td>26-Apr</td>
<td>57295BD</td>
<td>&gt; 5 days old</td>
<td>HMW, ++</td>
<td>DM</td>
<td>yes</td>
</tr>
<tr>
<td>26-Apr</td>
<td>57297BD</td>
<td>&gt; 5 days old</td>
<td>HMW, ++</td>
<td>FRAX</td>
<td>yes</td>
</tr>
<tr>
<td>26-Apr</td>
<td>57319BD</td>
<td>7 days old</td>
<td>HMW, ++</td>
<td>FRAX</td>
<td>yes</td>
</tr>
<tr>
<td>26-Apr</td>
<td>57320BD</td>
<td>8 days old</td>
<td>HMW, ++</td>
<td>FRAX</td>
<td>yes</td>
</tr>
<tr>
<td>26-Apr</td>
<td>57321BD</td>
<td>5 days old</td>
<td>HMW, ++</td>
<td>FRAX</td>
<td>yes</td>
</tr>
<tr>
<td>26-Apr</td>
<td>57317BD</td>
<td>5 days old</td>
<td>HMW, ++</td>
<td>FRAX</td>
<td>yes</td>
</tr>
<tr>
<td>5-May</td>
<td>57439BD</td>
<td>clotted, very dirty</td>
<td>degraded</td>
<td>Bank</td>
<td>new sample requested</td>
</tr>
<tr>
<td>13-May</td>
<td>57584BD</td>
<td>hemolyzed, clotted, no buffy? Prot K o/n @ 50</td>
<td>HMW</td>
<td>FRAX</td>
<td>yes</td>
</tr>
</tbody>
</table>

HMW - high molecular weight  
++ - some degradation observed  
+ - slight degradation observed
Appendix F – “Rejection” Report Template

MOLECULAR GENETICS DIAGNOSTIC LABORATORY
Children’s Hospital of Eastern Ontario
401 Smyth Road, Room W3423
Ottawa, Ontario K1H 8L1
Tel: (613) 738-3230
Fax: (613) 738-4814

Date Sample Drawn:
Date Sample Received:
Date of Report:
CHEO Pedigree No.:
Lab #:

Referring Diagnosis:

Referred by:
Address:

<table>
<thead>
<tr>
<th>Birth Date</th>
<th>Name</th>
<th>Ped No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>day/mo/yr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This sample was not processed as it did not meet our criteria for acceptance.

Reasons for rejection:

☐ Test not performed in this laboratory
☐ Sample not received in an EDTA tube
☐ Sample is older than 3 days (FSHD and MD2) or 7 days (other tests) upon receipt
☐ Insufficient volume
☐ Clotted blood
☐ Mislabeled/unlabelled tube
☐ Inadequate DNA ___________
☐ Other ___________

Suboptimal conditions observed:

☐ Sample not received at room temperature
☐ Sample hemolyzed
☐ Insufficient volume
☐ Other ___________

If this test is still required, please submit an additional peripheral blood sample in an EDTA tube and with an appropriate requisition. Please refer to our website for detailed description of sample requirements.

If this test is still required, please contact an appropriate laboratory regarding their sample requirements.
Appendix G – “Sub-optimal” Preliminary Report Template

MOLeCULAR GENETICS DIAGNOSTIC LABORATORY
Children’s Hospital of Eastern Ontario
401 Smyth Road, Room W3423
Ottawa, Ontario K1H 8L1
Tel: (613) 738-3230
Fax: (613) 738-4814

Date Sample Drawn:
Date Sample Received:
Date of Report:
CHEO Pedigree No.:
Lab #:

Referring Diagnosis:

Referrer by:
Address:

<table>
<thead>
<tr>
<th>Birth Date</th>
<th>Name</th>
<th>Ped No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>day/mo/yr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please be advised that the condition of the sample above was suboptimal upon receipt (see reasons below). We are proceeding with the requested test but we are unsure how this will impact testing. We will contact you if an additional sample is required.

Suboptimal conditions observed:

☐ Sample not received at room temperature
☐ Sample hemolized
☐ Insufficient volume
☐ Sample is older than 3 days (FSHD/MD2) or 7 days (other tests) upon receipt
☐ Other

Rejection criteria:

☐ Test not performed in this laboratory
☐ Sample not received in an EDTA tube
☐ Insufficient volume
☐ Clotted blood
☐ Mislabeled/unlabelled tube
☐ Inadequate DNA
☐ Other

Please refer to our website for detailed description of sample requirements.
Appendix H: Retrospective analysis of DNA quality – 6 months of storage

a) Figure H-1 a) Integrity gel of samples extracted from January 6-13, 2011. b) Examination of DNA Integrity for same samples as gel to the left. All samples on gel b were electrophoresed approximately 6 months post-extraction.

Figure H-2: Comparison of DNA concentrations 6 month old samples in Figures H1) a and b) based on optical density measurements at the original and 6 month intervals.
Appendix I - Retrospective analysis of DNA quality – 12 months of storage

Figure I-1: Integrity gel originally run June 14, 2010

Figure I-2: 12 month stability check of samples from June 14, 2010.

Figure I-3: Concentrations from the 12 month stability check from Figure I-1 and I-2.
### Appendix J – Adoption of Laboratory Quality Indicators

<table>
<thead>
<tr>
<th>Quality Indicator</th>
<th>Recommendation / Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of new tests deployed</td>
<td>Tracking of numbers recommended</td>
</tr>
<tr>
<td>Number of patients tested</td>
<td>Currently tracked through workload and departmental statistic packages</td>
</tr>
<tr>
<td>Number of tests performed/outsourced</td>
<td>Tracking of data recommended</td>
</tr>
<tr>
<td>TAT – from receipt to reporting</td>
<td>Currently tracked</td>
</tr>
<tr>
<td>EQA</td>
<td>Currently done for all tests</td>
</tr>
<tr>
<td>Test failures</td>
<td>Refer to section entitled “Development of a reporting procedure for rejected and sub-optimal samples”</td>
</tr>
<tr>
<td>Complaints</td>
<td>Currently managed through AEMS reporting system</td>
</tr>
<tr>
<td>Complaint response times</td>
<td>Currently managed through AEMS reporting system</td>
</tr>
<tr>
<td>Customer satisfaction survey</td>
<td>In progress (through EORLA)</td>
</tr>
<tr>
<td>Analytical non conformities</td>
<td>Currently managed through AEMS reporting system</td>
</tr>
<tr>
<td>Outcomes of EQA</td>
<td>Currently tracked</td>
</tr>
<tr>
<td>Outcomes of internal audits</td>
<td>Currently tracked</td>
</tr>
<tr>
<td>Document control of new and revised protocols or policies</td>
<td>Currently done through Paradigm</td>
</tr>
<tr>
<td>Corrective action completion</td>
<td>More complete documentation of CQIs recommended</td>
</tr>
<tr>
<td>Unplanned absences</td>
<td>Refer to section entitled “Protocol Refinement”</td>
</tr>
<tr>
<td>Robustness of tests</td>
<td>Currently tracked through QC</td>
</tr>
<tr>
<td>Number of Reporting Mistakes</td>
<td>Tracking of revised reports recommended (ie. through new LIS program)</td>
</tr>
<tr>
<td>Retrospective examination of test performance for sub-optimal samples</td>
<td>Refer to section entitled “Retrospective examination of test performance for sub-optimal samples”</td>
</tr>
</tbody>
</table>
Appendix K – Optimization of Integrity Gel Conditions

Figure K-1: 1% Agarose gel made with 0.5% TBE run for 45 minutes at 100 volts. Lane 1: 2ul of HindIII lambda marker. 1ug of DNA sample was loaded into each lane.

Figure K-2: 1% Agarose gel made with 0.5% TBE buffer run at 75 volts for 1.5 hours. Lane 1: 250ng of HindIII lambda marker. 50 ng of DNA was loaded into all other lanes.

Figure K-3: 1% Agarose Gel made with 0.5% TBE run at 75 volts for 60 minutes. Lane 1: 50ng of HindIII lambda marker. 50ng of DNA was loaded into each well.
Appendix L: Flowchart for 3-week and 1-year Integrity Gels

DNA extracted & OD readings

Weekly Integrity Gel

- DNA tested in-house & stored at 4°C for 6 months
- Bank DNA placed in “3 week Integrity Gel” box at 4°C
- An aliquot of Banked sample sent to outside lab for testing; remainder is stored

At 3 week mark, the OD is re-measured and samples are electrophoresed

Sample held in 4°C storage with all other DNA samples

“Send-out” DNA is placed in 12 month check box sorted by month of extraction and stored at 4°C

At 12 month mark, the OD is re-measured and samples are electrophoresed
Appendix M – Report from the DNA Quality Task Force External Review

External Review

CHEO Molecular Genetics Diagnostic Laboratory (MGDL)
DNA Quality Task Force Investigation

September 12, 2011

Dr. Martin Somerville, PhD, FCCMG, FACMG
Director, Genetic Laboratory Services
Alberta Health Services
Professor, Department of Medical Genetics
University of Alberta
Phone: 780 407-7614
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1. Scope of the Review
The external review of the MGDL Quality Task Force investigation was undertaken to provide an outside opinion on the activities and findings of the Task Force. This review was carried out on a total of 64 files that were received from the Task Force including, but not limited to, meeting agendas and minutes, laboratory protocols, laboratory data, and a draft report. The reviewer was contracted to:

- Review current protocols related to sample receipt, DNA extraction and DNA storage to identify any deficiencies in the protocols that may negatively impact DNA quality.
- Review quality measures collected by the lab over the last year to assess extent of variability in DNA quality over that time period and identify possible trends that may have contributed to reduced DNA quality.
- Propose recommendations for collection of additional DNA quality indicators that are not currently collected, if applicable.
- Review DNA task force meeting minutes (and any associated attachments) and make recommendations for any further investigations that may not have been considered by the task force.
- Provide critical assessment of the final report prepared by the task force prior to its release.

It should be noted that the utility of this review is limited by the reviewer’s interpretation of documentation that was examined. This may result in inconsistencies between this reviewer’s understanding of current policies and practices relative to policies and practices that are actually in place. It should also be noted that this review has been completed subsequent to the release of the Task Force Report.
2. Review of Current Protocols

Current protocols for sample receipt, DNA extraction, and DNA storage were examined.

I. Sample Receipt

The MGDL Specimen Receiving protocol for blood (version 1.1) provided to the reviewer, was approved by the Head of the laboratory on January 25, 2010. The protocol overall is fairly comprehensive and addresses contingencies that could reasonably be expected within the laboratory. Some gaps were noted, one of which could affect DNA quality.

Specifically, although limits are provided for acceptable transit times (interval between collection and receipt within the laboratory), there is no information on when specimen processing should be initiated, or how the specimen should be stored prior to processing within the laboratory. A specimen could remain unprocessed at any temperature within the laboratory for an extended period of time without contravening the protocols for receipt or DNA extraction. The MGDL does have a Sample Extraction Priority protocol (v. 1.0), approved August 8, 2008, but this does not specify what the interval of 3-7 days old for blood samples refers to, it is also not clear in this protocol what is meant by “Bloods (1-2 olds).”

A second gap in the specimen receiving protocol is the lack of a clear indication as to what, if any, of the required information on a laboratory requisition and/or specimen container is mandatory upon sample receipt. The protocol states that for a sample to be accepted, a follow-up is required to obtain missing information. However, there is no requirement to verify that identifiers on the specimen container are consistent with identifiers on the accompanying requisition. This is identified in the Task Force minutes of July 21, 2011 as a deficiency that would warrant rejection of the specimen, but is not addressed in the Specimen Receiving Protocol that was provided for review.

There is also no requirement for the presence upon specimen receipt of a minimum data set, such as unique identifiers. Although highly unlikely, a specimen could arrive without a patient name, date of birth, or health card number on the requisition, blood tube, or both and still be accepted by the lab if this information is obtained by other means. This would significantly compromise the integrity of identity verification for the specimen, but it should be noted that this should not directly affect DNA quality.

The laboratory would benefit from a policy that requires verification of identifiers for any patient specimen transferred manually from one container to another. This is typically based upon visual verification by a second member of the lab staff and is referred to as a rack check. Rack checks are included in the protocol for the DNA extraction procedure, but it would be beneficial to have this requirement clearly stated as a global policy for all transfers of patient material within the laboratory.

II. DNA Extraction

The MGDL ArchivePure DNA extraction protocol (version 1.2.3) provided to the reviewer, was approved by the Head of the laboratory on May 5, 2011. Task Force minutes from the meetings dated June 16 and June 30, 2011 state that a draft version of the protocol being used was not yet current on the Paradigm system. Paradigm is presumed to be a document information system in use in the laboratory. It is therefore, not clear as to when this version of the protocol was implemented as a standard operating procedure in the laboratory. Some minor discrepancies are apparent between v. 1.2.3 of the MGDL protocol and the manufacturer’s recommended protocol. These are primarily omissions of detail provided by the manufacturer such as ensuring that vortexing is performed “vigorously” or at “high speed,” or specifying the amount of time required for decanting sample supernatant, or the number of repetitions of tube inversions required for DNA
precipitation. Some more significant discrepancies were noted including the recommended temperature for cell lysate incubation (manufacturer recommends 37°C , but MGDL protocol requires 55°C), and recommended incubation time for DNA hydration (manufacturer recommends 1 hour at 65°C, but MGDL protocol requires 2 hours). In addition, the MGDL protocol for DNA pellet washing has a higher risk in resulting in sample loss than the manufacturer's recommendation, as the MGDL protocol requires active pellet re-suspension for initial wash which could result in loss of pellet during decanting on the supernatant. However, it is not clear that any of these variations between the MGDL protocol and the manufacturer’s recommended protocol will result in significantly increased risk of sample degradation.

III. DNA Storage
Based on documentation provided, DNA storage conditions should not compromise quality. Samples are stored in DNA Hydration Solution (assumed to be a standard 10mM Tris, 1mM EDTA solution at pH 8.0) at 4°C. These storage conditions are fairly universal, and although some laboratories store DNA at -20°C, 4°C storage is common practice and does not actively promote degradation of DNA.

3. Review of Quality Measures
The task force collected data on key measures that may contribute to the quality of extracted DNA. These measures included criteria that affect specimen integrity such as elapsed time from collection to receipt in the lab, and specific variables such as collection site and the identity of the MGDL technologist that performed the extraction. In addition, source and lot numbers for extraction reagents were examined for potential correlation with extract quality. This review was comprehensive, out of necessity, as there are numerous variables that could have relevance.

The task force conducted a walk-through of the space to identify any potential problems with reagent and specimen storage and handling. In addition, the activities of technologists were compared to the MGDL extraction protocol, during direct observation by a member of the task force. Both initiatives identified potential problems that could compromise the quality of DNA extracts.

One factor that did not appear to be considered was the number of specimens that were processed at one time. Peripheral blood samples are routinely extracted by one of three methods, manually using a commercial kit (ArchivePure), manually with in-house reagents (agarose gel plugs), or on an automated system using commercial reagents (NucliSENS easyMag Extractor). All of the quality control problems in the lab appear to result only from manual extractions using the Archive Pure kit. The MGDL receives approximately 4,600 specimens per year, which would result in an average of approximately 18 specimens per day (assuming a five-day work week) that require processing. No information was provided on the relative number of specimens that require manual extraction with the ArchivePure kit, but this would presumably account for a significant proportion of the total. It is also not clear whether the same individual is responsible for all extraction procedures each day, which could result in activities being split between as many as three different methods. Any increased workload may correlate with reduced quality of DNA as time-sensitive steps in the extraction process may be less consistent between specimens.

4. Additional DNA quality indicators
The quality indicators that are currently collected by the laboratory (optical density at 260 and 280 nm, and electrophoretic integrity) are sufficient to demonstrate DNA quality. The MGDL has proposed re-examination of stored extracts at 1 week, 3 week, and 1 year intervals to monitor DNA integrity over time. A more stringent method for ensuring DNA integrity would involve prolonged incubation of extracts at a temperature that would promote degradation if contaminants are present. Specifically, aliquots of DNA extracts could be incubated at 37°C for 2-4 days prior to analysis. If DNA is still intact after this incubation, prolonged storage at 4°C would not be expected to result in any further loss of integrity. This would have the benefit of more immediate
feedback regarding any problems with DNA quality, as opposed to discovering a problem a minimum of 1 week after an extraction. It could also serve to test new reagent lots before using them in routine extractions.

5. Review of Task Force Meeting Minutes
Minutes were obtained for a total of 8 DNA Quality Task Force meetings dated: May 2, May 11, June 9, June 16, June 23, June 30, July 14, and July 21.

Key initiatives that were developed include the establishment of a focus group to maximize input of ideas and a mechanism for organizing and prioritizing task force activities. Several needs were identified including the establishment, and adherence to, clear specimen acceptance/rejection criteria and the establishment of defined DNA quality measures. Minutes also document the finding of inconsistencies observed in extraction procedures between technologists and/or technicians. A solution to this problem has been found whereby, an additional Medical Laboratory Assistant has been hired to perform DNA extractions. This increases the FTE commitment dedicated to extractions to 2.0, and eliminates contingencies in which coverage of this procedure by technologists is necessary. However, the additional position is temporary and so inconsistency in DNA extraction procedures has the potential to return if this position is not funded on an ongoing basis.

Additional variables that were considered, but which did not correlate with DNA quality: date of extraction, reagent lot number, sample quality on receipt, loss of integrity in samples processed in parallel. Variation in lot-to-lot of ArchivePure reagents may correlate with DNA quality but this was unresolved in the Task Force minutes. The task force determined that the alcohol (ethanol and isopropanol) that had been obtained from a third party supplier for DNA precipitation was not of suitable quality. A source of molecular grade alcohol was identified and a task force member was assigned responsibility for placing an order. However, there was uncertainty as to whether or not the supplier of molecular grade ethanol is licensed to sell this product in Ontario. The task force will validate these reagents with the use of standard DNA quality indicators (optical density and electrophoretic integrity of extracts produced).

A deficiency in outcome measurement was noted by the task force: the MGDL does not routinely request, or receive, copies of reports from laboratories used for referred-out testing. This is required by accrediting bodies in some jurisdictions and provides the lab with information such as sensitivity and specificity of referred-out tests, turn-around-time for referred-out tests, and mutation status in families for which other testing may be required. The MGDL would also receive reports of DNA quality problems directly from the referred-out laboratory. For these reasons, this practice is highly recommended.

6. Assessment of Final Report
The Final Report is detailed and comprehensive and provides fairly complete documentation of Task Force activities. It would benefit by including an executive summary to highlight the key issues that were identified along with recommendations. However, this review has been completed after release of the Task Force Report limiting the utility of assessment.

7. Recommendations
I. Sample receipt protocols should clearly state acceptable time and temperature limits for specimen storage in the laboratory prior to extraction.

II. The laboratory should establish a minimum data set required (e.g., two unique identifiers) for specimen acceptance.
III. The laboratory should have a policy for specimen acceptance requiring that identifiers on the specimen container are consistent with identifiers on the accompanying requisition.

IV. The laboratory should have a policy stating that identifiers for all manual transfers of patient material between containers within the laboratory are verified by another qualified staff member.

V. The laboratory should develop and implement an extraction protocol that is consistent with manufacturer’s recommendations. The MGDL should have a mechanism in place to verify that all staff performing the extraction procedure, have read, understood, and will use this extraction protocol.

VI. The task force should consider the possibility that there was a correlation between the number of specimens that were processed at one time and DNA quality.

VII. The laboratory should establish a minimum rotation period of one week for any technologist that is participating in coverage for extraction over an extended period of time. This time should be dedicated as much as possible to extraction procedures and related activities only.

VIII. The laboratory should either provide ongoing funding for the second Medical Laboratory Assistant position, or allocate capital funds for an appropriate automated extraction system in order to ensure a greater level of consistency.

IX. The laboratory should consider more rapid methods of determining DNA quality, such as the incubation of sample aliquots at 37°C prior to testing integrity.

X. The laboratory should have a policy that referred-out laboratories are required to send copies of reports directly to the MGDL.