

**Matrix DNA Diagnostics – Tulane University**  
**Patient Consent form – COL1A1/COL1A2 Mutation assay**  
**Detection of Mutations in the Collagen I genes (COL1A1, COL1A2) Causing Osteogenesis Imperfecta and Related Disorders**

**BACKGROUND** Collagen I is a tough, fibrous protein that provides a major part of the strength of bones. Mutations in the two genes that code for collagen I (COL1A1, COL1A2) cause either decreased synthesis of the protein or cause synthesis of defective forms of the protein. The result is weak or fragile bones. Mutations in these two genes have been found in over 90% of the patients with Osteogenesis Imperfecta and have been shown to be the cause of the disease in these patients. The DNA testing carried out in our laboratory will detect most, but not all, of the mutations that can be found in the two genes for collagen I, and that can cause Osteogenesis Imperfecta and related disorders. The COL1A1 and COL1A2 genes are among the more complex of human genes. Therefore, complete analysis of the genes for the presence of mutations requires a large number of careful manipulations and analyses of the DNA. Thus, completion of the test may take two to four weeks.

**TEST LIMITATIONS** The analyses may fail to detect a few mutations in patients who have mutations in the genes as a cause of the disease. We do not have an accurate estimate of how many mutations are missed, but our best estimate is that >90% are in fact detected. Also, some changes in the gene structure can be difficult to interpret in terms of whether they cause the disease or are more neutral changes. Also, mutations in other genes are not detected. However, in the vast majority of patients with Osteogenesis Imperfecta, the test will detect a mutation and provide definitive information that the mutation causes the disease.

**SAMPLE** The DNA test requires extraction of DNA from the patient's blood. The DNA can also be extracted from a tissue sample, such as a small piece of skin, but this is usually less convenient. Most commonly, blood is drawn by trained person designated by your physician. The blood is sent by overnight courier to the laboratory. There, the DNA is extracted and over several weeks the analysis is performed. The result is sent in written form to the physician who will relay it to the patient or relative designated to receive the result. The results are confidential and will not be released to any institution or individual without your written consent.

**STORAGE** In most instances not all the DNA is consumed in the analyses. The remaining DNA will be stored in this laboratory for two years, after which the DNA sample will be discarded without informing the patient or family members. The DNA will not be released to any institution or individual without your written consent. The DNA may be used at the discretion of the laboratory supervisor for research purposes, under an anonymous label. The results of the research studies will not be reported to the patient or his physician at any time, nor will the laboratory assume any responsibility of the research studies on these specimens.

**LABORATORY** The laboratory is an up-to-date molecular genetics facility and uses the most current techniques clinically practical to perform the analysis. The techniques used may change to become more sensitive or specific in the future. In such cases, at the request of your physician, the test may be rerun on your sample, if clinically indicated.

**LEGAL ISSUES** The laboratory assumes no responsibility for injury or illness resulting from the drawing of blood or removal of tissue samples from the patient. The laboratory also assumes no responsibility for liability or loss incurred as a result of the outcome of this test. The laboratory assumes no responsibility for the mislabeling or misidentification of submitted patient specimens and assumes all submitted patient data to be correct as listed in the submission form.

In signing this form, I indicate that I understand the information presented above and agree to these stipulations.

E T E L E P M O C

<b>Patient Signature</b>	<b>Date</b>	<b>Witness Signature</b>	<b>Date</b>

For any questions, please do not hesitate to call us at (504) 988-7706



**FORM 1- Instructions for submission of specimen for DNA testing**

**The patient should be fully informed about the test.**

**Nature of the test:** The test detects mutations in gene(s) involved in the synthesis of proteins of connective tissue. Extraction of DNA from blood or tissue of the affected individual is required.

**Test limitations:** Only the gene implicated in the disorder will be studied which your physician will determine. Mutations in other genes will not be detected. The rate of mutation detection varies with the disorder and the gene studied. Mutations in non-structural portions of the gene will generally not be detected.

**Test results:** The test results are reported to the physician in writing. The test generally takes about 2-4 weeks; we try to expedite the analysis for prenatal testing.

**Sample requirements**

•**Whole Blood:** 10 cc (adults) in purple top (EDTA) tube should be drawn by venipuncture. In the case of small children, 3-5cc of EDTA blood is generally sufficient amount to complete the analysis. There are no special dietary or blood drawing considerations. Patient must not have received a transfusion recently. Heparin inhibits the PCR reaction, therefore heparin tubes are not acceptable. DNA extracted from heparin-contaminated blood is not accepted

•**Fibroblasts, amniocytes, CVS:** Preferably 2-4 confluent T25-flasks of cultured cells. It is highly advisable to call prior to submitting cultured cells.

•**Tissues:** Please call the lab at 504-988-7706. We do not take whole tissue or paraffin blocks. We do accept extracted DNA from tissues, but not from paraffin blocks.

**Sample submission**

Sample should be shipped by **overnight courier, preferably Federal Express** (Use DHL/Airborne with caution as we have experienced significant delivery issues with them). Please send with a cold pack during summer months. If blood sample can't be shipped the same day, please store at +4C until shipment. All tubes/flasks should be labeled with patient name and date of birth, or other identifying criteria. Please do not send any samples on Friday. **Patient Information Form** (Form 2), **Payment Information Form** (Form 3) and an **OPTIONAL signed Patient Consent Form** should accompany the sample. If laboratory fee is paid by check that accompanies the sample, Form 3 is not needed. Check should be made out to **Tulane University**. We can assist with test description and related information for insurance companies.

<b>Charges</b>	
<b>Osteogenesis Imperfecta:</b> Collagen I Gene (COL1A1 and COL1A2) Analysis	\$1800
<b>Chondrodysplasias:</b> Collagen II Gene (COL2A1) Analysis	\$1400
<b>Stickler/Marshall Syndrome:</b> Analysis of the COL2A1 and COL11A1 Genes	\$2300
<b>Marfan Syndrome:</b> Fibrillin -1 gene (FBN1) Analysis	\$1400
<b>Metaphyseal dysplasia type Schmid:</b> Collagen X gene (COL10A1) Analysis	\$460
<b>Known mutation (family member or prenatal)</b> in any of the above	\$350

**Sample and Paperwork should be sent to:** Please call (504) 988-7706 before submitting a sample for the first time **if you need assistance.**

Matrix DNA Diagnostics  
 Attn: DNA Diagnostics Laboratory  
 Center for Gene Therapy  
 Tulane University Health Science Center  
 1430 Tulane Avenue, TB 28  
 Tidewater 2140  
 New Orleans, LA 70112



<b>Patient name:</b>	<b>Date of Birth:</b>
<b>Hospital Reference Number:</b>	<b>SSN:</b>
<b>Address:</b>	<b>Telephone:</b>

**Patient Diagnosis/Clinical information:**

**Required attachments:**

- copy of a driving license for the policy holder, if insurance bill
- copy of clinical history and physical examination

<b>Check for the test requested:</b>	<input type="checkbox"/>	COL1A1/COL1A2 gene analysis for Osteogenesis Imperfecta
	<input type="checkbox"/>	COL2A1 gene analysis for SED, ACGII, HCG, Kniest dysplasia
	<input type="checkbox"/>	COL2A1 /COL11A1 gene analysis for Stickler/Marshall Syndrome
	<input type="checkbox"/>	FBN1 gene analysis for Marfan Syndrome
	<input type="checkbox"/>	COL10A1 gene analysis for Schmid Metaphyseal dysplasia
Proband name must be provided in box above.	<input type="checkbox"/>	Known Mutation (family member or prenatal) in any of the above

<b>Type of specimen:</b>	<b>Is this a prenatal test YES NO</b>
<b>Collected by:</b>	<b>Collection date:</b>

<b>Referring Physician Name</b>	<b>Contact person</b>
<b>Telephone Fax</b>	<b>Address</b>
<b>Request Date</b>	<b>Physician's Authorized Signature</b>

**If this is a known family mutation, please provide the proband's name or the proband report.**

**OI feature check list – check all that apply**

- Easy Bruising
- Blue Sclera
- Dentinogenesis Imperfecta
- Fractures in Childhood
  - <10
  - >10
- Fractures in Adulthood
  - <10
  - >10
- Hearing Loss
- Bowing of the bones

### Form 3: Payment Information/Invoice

**1. Payment by check.** Please make the check payable to **Tulane University** and send to:

Matrix DNA Diagnostics  
Center for Gene Therapy  
1430 Tulane Ave, SL-99  
New Orleans, LA 70112

<b>ICD-9 code:</b>	756.5	<b>Tax/Fed ID#:</b>	72-0423889	<b>CLIA#:</b>	39D0903989
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**2. Payment by Institution.**

Contact Person:	Phone number	Bill to- address
	Fax number	

**3. Payment by Insurance Company. ATTACH A READABLEPHOTOCOPY OF THE INSURANCE CARD (BOTH SIDES)! CARDHOLDER INFORMATION MUST ALSO BE INCLUDED (address and date of birth)!**

Name of Policy Holder:	SSN or member#:	Plan/Group#:
Insurance Provider's Name:	Phone Number:	Address:
Claim Dept's phone #:	Insurance Contact person:	Pre-approval number:

**DISCLAIMER:** DNA Diagnostics may not be an in-network provider with most insurance companies. We reserve the right to decline/refuse any insurance as a payment option. The patient accepts full responsibility for any payment not covered by insurance. **Patients may be required to provide alternate funding, upon request, in advance for any amount that may not be covered by insurance. WE DO NOT ACCEPT ANY MEDICAID.**

COL1A1 and COL1A2 for Osteogenesis Imperfecta

CPT codes	Description	# Performed	Cost/test	Extended
83890	DNA isolation	1	\$10.00	\$10.00
83898	Polymerase chain reaction	38	\$10.00	\$380.00
83904	Nucleic acid probe w/ sequencing	35	\$40.00	\$1400.00
83912	Interpretation and report	1	\$10.00	\$10.00
<b>TOTAL COST</b>				<b>\$ 1800.00</b>

\*Please note that the COL1A1/COL1A2 analysis is a multistep test, which involves sequencing the entire coding region of two very large genes and, therefore, involves multiple tests to isolate the genes, identify the genes, and sequence all coding regions in every patient. For more information, please call the lab about the test (504-988-7706)

<b>In signing this form, I indicate that I understand the information presented above and agree to these stipulations.</b>	
<b>Patient Signature</b>	<b>Date</b>

## ACCURACY AND SENSITIVITY OF DNA ASSAY (PCR-SEQUENCING) MUTATIONS CAUSING OSTEOGENESIS IMPERFECTA

Center for Gene Therapy Tulane University Medical School

### The Assay:

Our assay involves two steps:

- (a) **amplifying** DNA sequences from all 51 exons and exon flanking regions for the COL1A1 gene, and from 47 of the 52 exons and exon flanking regions for the COL1A2 genes (the collagenous domain and the C-propeptide) for human type I procollagen using 100 specific primer pairs for the polymerase chain reaction (PCR);
- (b) nucleotide **sequencing and analysis** of all PCR products.

### Sensitivity Assay:

In preliminary tests of our assay, we asked the question: "Will the assay detect all the mutations that have been previously found in the genes for type I procollagen and similar genes?" We assayed genes that were previously shown to contain 78 different single base mutations. We detected 77 or 98.7%.

Our further data on the sensitivity of the assay came from analyses we have carried out on DNA samples (blood or fibroblasts) sent to us by physicians or geneticists who made the diagnosis of OI or "probable OI" on one or more patients (see Table I).

### Interpretations of the Test Results:

Most patients and families with OI have "private mutations" in the sense that the same mutation is rarely found in more than one unrelated family. Hence the need for analysis of about 15,000 base pairs from each patient to detect a mutation. Fortunately, however, the base sequences of the two genes for type I procollagen (COL1A1 and COL1A2) are highly conserved and there are few neutral variations that change a codon for an amino acid or a consensus site for RNA splicing of an exon in normal individuals. The mutations fall into three general classes:

- (I) Most patients with severe forms of OI have single-base substitutions that change a codon for glycine, the smallest amino acid, to a codon for a bulkier amino acid that distorts the shape (conformation) of the rod-like collagen molecule.
- (II) Most patients with the mildest form of OI (type I) have single-base substitutions or larger changes that either introduce premature termination codons for translation of the messenger-RNA or alter consensus sites for RNA splicing out of introns. Mutations in classes I and II are relatively easy to interpret as causing OI because they have now been seen in large numbers of patients and families and because molecular consequences of many of them have been defined in great detail.
- (III) A few patients have mutations that change codons for amino acids other than glycine or change intron sequences that may or may not be required for normal RNA splicing. Class III mutations are difficult to interpret. Frequently, it is necessary to find the same mutation in another member of the same family who has OI in order to make a diagnosis. Fortunately, the number of class III sequences is decreasing, as our database gets larger.

### **Predicting the Severity of the Disease:**

Most mutations that introduce premature termination codons or alter consensus sites for RNA splicing of introns in the genes for type I procollagen produce the mildest form of OI referred to as type I. Single-base amino acid substitutions that convert codons for glycine for bulkier amino acids produce forms of OI that vary from relatively mild to lethal. Unfortunately, it is still difficult to predict the severity of OI from the precise information that can be generated about a mutation. The best guide is the severity of the same disease in another member of the same family who also has the same mutation. Even this guide, however, can be misleading in that there are rare instances in which one member of the family with the mutation has a severe form of OI whereas the other member of the same family with the same mutation has a mild form.

### **Mutations Causing OI that Cannot Be Detected by the DNA Assay:**

The assay will not detect mutations that alter promoter sequences, enhancer sequences, or repressor sequences of the type I collagen genes. Such sequences have not been accurately enough defined and they show large variations between one normal individual and another. However, mutations in such sequences appear to be a very rare cause of OI.

The test will also not detect mutations in genes other than the two genes for type I procollagen that may cause the same or similar signs and symptoms as those seen in typical patients with OI. Recent reports suggest that rare patients with OI in fact have mutations in other genes that are still not defined.

Table I: Results of Detection of Mutations Causing Osteogenesis Imperfecta by PCR/CSGE/Sequencing (Data as of Oct. 18, 1999)

<b>Criteria for Clinical Diagnosis</b>						<b>DNA assays</b>		
<b>OI Type</b>	<b>Bone fragility</b>	<b>Short stature and deformities</b>	<b>Blue Sclerae</b>	<b>Abnormal Teeth</b>	<b>Hearing Loss</b>	<b>Samples assayed</b>	<b>Mutations found</b>	<b>% Positive</b>
I	Mild Mild	Absent Absent	Present Absent	May be Present May be Present	One-half May be Present	47 15	44 9	94% 60%
II (lethal)	Extreme	Severe	Present	?	?	30	26	87%
III	Severe	Progressive deformities	Bluish at birth	Frequently	Frequently	21	17	81%
IV	Variable	Mild to Moderate	Absent	May be Present	Frequently	17	13	76%

### **NOTES:**

(1) All clinical diagnoses of OI were made by referring physicians and geneticists. The clinical diagnoses were not verified by us. Therefore, some of the patients may have been incorrectly diagnosed, and the true percentage positive assays may be higher than the values here indicates.

(2) Because we have not detected a mutation in every patient with a clinical diagnosis of OI, the test cannot be used to exclude OI, i.e. a negative test cannot be used to conclude a patient does not have OI.