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November 2010

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Changes in Maternal Serum Screen Tests

Maternal Serum Screening tests are very useful to help identify pregnancies of increased risk for Down syndrome (DS), Trisomy 18 (T18), or open neural tube defects (ONTD) such as spina bifida. The intent of these screening tests is to enable pregnant women to make informed decisions regarding the pregnancy and be better prepared in the event of the birth of an affected infant.

Down syndrome is the most common chromosome abnormality among live births and the most frequent form of intellectual disability caused by a demonstrable chromosomal aberration. It arises from the presence of an extra copy of chromosome 21 in the cells of the fetus. Mental retardation, characteristic features of the face and medical problems such as heart defects occur as a result of this extra chromosome 21. About 40% of pregnancies with Down syndrome will miscarry between 11 weeks and term, but 9 out of 10 affected babies who reach term will survive the first year and about one half of these individuals will reach 60 years of age. The syndrome is characterized by moderate to severe learning disability (average IQ approximately 40) in combination with short stature, characteristic facial features, heart defects (40 to 50% of cases), intestinal malformations (10% of cases), problems with vision and hearing (50% of cases), an increased frequency of infection, and other health problems.



Photo: Kevin Vance

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Maternal age alone was the initial risk factor used for screening pregnancies for Down syndrome. The risk of giving birth to a baby with Down syndrome as a function of maternal age is nonlinear and ranges from approximately 1 in 1500 in young women to 1 in 10 in a 48-year-old. The risk is almost constant at ages 15 to 25, rises slowly between ages 25 to 35, and then increases approximately four-fold from ages 35 to 40 and 10-fold from ages 40 to 45; there are data that suggest the risk of Down syndrome does not increase further beyond age 45.

T18 is a rare (birth prevalence about 1 in 7000) and usually fatal abnormality which arises from an extra copy of chromosome #18 in the cells of the fetus. The prevalence is 3-5 fold higher in the first and second trimesters than at birth because many affected fetuses die in utero with advancing gestation. Trisomy-18 causes severe mental retardation and birth defects. Only 10% of live borns with this condition will reach one year of age.

ONTD's are congenital structural abnormalities of the brain (Anencephaly) and vertebral column (spina bifida) that occur either as an isolated malformation, along with other malformations, or as part of a genetic syndrome. The cause of non-syndromic ONTD's is not known. Scientists believe that genetic, environmental and nutritional components all play an important role. ONTD's occur in 1-2 per 1000 pregnancies and are the second most common major congenital anomaly worldwide. Anencephaly accounts for one half of all cases of ONTD and is incompatible with life. With treatment, 80-90% of infants with Spina Bifida survive with varying degrees of disability. Most importantly, ONTD's are among the few birth defects for which primary prevention is possible (*ACOG Practice bulletin 44:203*).

In 2007, the American College of Obstetricians and Gynecologists (ACOG) recommended that all women be offered aneuploidy screening before 20 weeks of gestation and that all women should have the option of invasive testing regardless of maternal age.

There are various screen tests available and choosing the most appropriate screening test can be confusing. Summarized below are the clinically relevant details of each test.

First Trimester Maternal Screen Test (MSFIRST)

- The first trimester screen test is optimally performed at 11 to 13 weeks of gestation.
- It involves sonographic determination of nuchal translucency (NT) and gestational age (by crown-rump length) combined with the serum markers pregnancy-associated plasma protein-A (PAPP-A) and human chorionic gonadotropin (HCG).
- This test does not screen for ONTD's, so second trimester screening for these defects still needs to be performed (Recommend MSAFP test).
- The results of DS and T18 screen are available in the first trimester. There is a higher risk for a positive result when the fetus does not have either disorder (false positive) compared to the Sequential or Integrated screen tests.
- This is the best screening test for women whose priority is privacy and early diagnosis; however, chorionic villus sampling (CVS), the diagnostic follow-up test, is associated with a higher risk of procedure related loss per woman screened than second trimester amniocentesis.

Sequential Screen Tests (MSSEQ1 and MSSEQ2)

- The Sequential Screen tests involve TWO blood draws, one in the first trimester and another in the second trimester as well as an ultrasound in the first trimester to measure the fetus' NT.
- The first trimester blood sample is tested for PAPP-A and total HCG.
- The second trimester blood sample is tested for Alpha fetoprotein (AFP), Total HCG, UE3 (Unconjugated Estriol) and Inhibin- A.
- The first trimester post test risk estimates will be provided ONLY if there is a high risk for DS or T18 (Greater than 1 in 25 cut off).
- If the first trimester test does not identify the pregnancy as being at increased risk, the post test risk estimates will be provided following the second blood draw in the second trimester. The second blood draw test results will be combined with the first trimester blood draw results to provide a final post test risk estimates.
- This testing has excellent detection capabilities for DS, T18 and ONTD's and a low chance of false positive test result.

Integrated Screen Tests (MSSINT1 and MSSINT2)

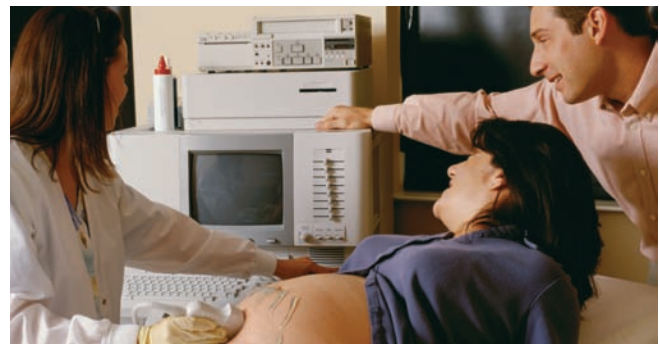
- The Integrated screening tests, like sequential tests as above involve TWO blood draws, one in the first trimester and another in the second trimester, with or without the inclusion of an NT measurement in the first trimester.
- The difference between the Sequential screen and the integrated screen is that the sequential screen provides first trimester results to women with a high risk for DS or T18.
- Since this test can be run with or without ultrasound measurement of NT, it provides an option to patients in areas where expertise in measurement of NT is not available.
- A disadvantage of the integrated test is that the patient has to wait until the second trimester to obtain her risk estimate.

A **positive screening test result** does not mean that a fetus definitely has a chromosome disorder, but only that an elevated risk has been detected. It is recommended that patients with a positive screen be referred for genetic counseling to discuss diagnostic and management options including fetal karyotype determination for definitive diagnosis. In the first trimester, karyotype is obtained by Chorionic villi sampling (CVS). In the second trimester, amniocentesis is performed to obtain amniocytes for chromosomal analysis. Analysis of the full karyotype is generally performed to allow detection of any aneuploidy (not just trisomy 21), as well as detection of major structural chromosomal abnormalities (eg, translocations, inversions, marker chromosomes). Patients who screen positive for ONTD's may be offered amniocentesis to measure the level of amniotic fluid AFP.

A **negative screening test result** means the patient's risk of having a baby with DS, T18 or ONTD is less than the specific cut-off level; it does not exclude the possibility of any of these defects, or that of another chromosomal disorder or congenital anomaly.

The below table shows the cut off and sensitivity and initial positive rates for the detection of Down syndrome.

Screen Test	DS Cut off	T18 Cut off	% Detection for DS	% False Positive for DS
MSFIRST	1 in 230	1 in 100	85	5-6
MSSEQ1	1 in 25	1 in 25	63	0.6
MSSEQ2	1 in 110	1 in 100	86 (Total)	1.6 (Total)
MSSINT1	NA	NA	NA	NA
MSSINT2	1 in 110	1 in 100	85 (Serum only) 87 (With NT)	3-4 (Serum only) 1.0 (With NT)



Effective, September 8, 2010, the following different maternal serum screen tests will be performed in house. There are no changes in specimen collection requirements, one full Gold/SST tube (Serum only). Specimen must be accompanied by a completed maternal screen requisition. These tests will be performed daily, Monday thru Friday, with a turn around time of two to four days.

Mnemonic	Test Name
MSFIRST:	Maternal Screen, First Trimester ONLY
MSSEQ1:	Maternal Screen, Sequential #1
MSSEQ2:	Maternal Screen, Sequential #2
MSSINT1:	Maternal Screen, Integrated #1
MSSINT2:	Maternal Screen, Integrated #2

MSFIRST Maternal Screen, First Trimester ONLY

- First Trimester (Between 11 weeks 0 days and 13 weeks 6 days).
- Includes PAPP-A and Total HCG.
- Requires NT (Performed by a certified ultrasonographer)
- CRL measurements must be between 4.2-7.9 cm.
- This test does not screen for ONTD (Open Neural tube defects).
- MSAFP test is recommended in 2nd trimester to screen for ONTD.

MSSEQ1: Maternal Screen, Sequential #1

- First Trimester (Between 11 weeks 0 days and 13 weeks 6 days).
- Includes PAPP-A and Total HCG.
- Requires NT (Performed by a certified ultrasonographer)
- CRL measurements must be between 4.2-7.9 cm.
- Patient test results will be released only without risk estimates, unless pregnancy is at high risk (Greater than 1 in 25) for DS and T18 in the first trimester.
- Patients who are at medium to low risk after the first draw should go for the second draw (MSSEQ2) to complete the screen.

MSSEQ2: Maternal Screen, Sequential #2

- Requires previously submitted first trimester (MSSEQ1) specimen.
- Second Trimester (Between 15 weeks 0 days and 22 weeks 6 days) Best time is 16-18 weeks of pregnancy.
- Includes AFP, Total HCG, UE3 and Inhibin- A.

MSSINT1: Maternal Screen, Integrated #1

- First Trimester (Between 10 weeks 3 days and 13 weeks 6 days).
- Includes PAPP-A
- An Ultrasound for NT is optional for this test (if performed, must be done by a certified ultrasonographer).
- CRL measurements must be between 3.6-7.9 cm.
- Patient test results (PAPP-A) and Best time to draw the second specimen will only be reported without any risk estimates.
- Second specimen (MSSINT2) must be drawn to complete the screen.

MSSINT2: Maternal Screen, Integrated #2

- Requires previously submitted first trimester (MSSINT1) specimen.
- Second Trimester (Between 15 weeks 0 days and 22 weeks 6 days).
- Includes AFP, Total HCG, UE3 and Inhibin- A.
- Final interpretative report is available only when the second specimen results are complete.

Test Requirements:

The blood must be sent to the laboratory along with a **completed maternal screen test requisition**. The test requisition must be filled out completely as test interpretation is dependent upon this information; therefore results may be delayed if information is not provided or missing. Maternal demographic information such as age, weight, gestational age, diabetic state and race is used together with the results of the serum screen tests in a mathematical model to derive a risk estimate for each condition to identify women at increased risk of having an affected child. Results will be reported as “Normal” or “Abnormal” along with “Pre-Test” and “Post-Test” risk for each condition.

For any questions regarding methodology and interpretations, please contact:

- Dr. L.V. Rao, Director of Core Laboratories at 774-442-9615 or via email at Lokinendi.Rao@umassmemorial.org
- Ms. Melissa Brown, Lead Technologist of Maternal Screening at 774-442-9636 or via email at Melissa.Brown2@umassmemorial.org
- Ms. Judy Barron, Manager of Automated Chemistry at 774-442-9616 or via email at Judy.Barron@umassmemorial.org
- Dr. Nichole Korpi-Steiner, Associate Director of Core Laboratories at 774-442-9634 or via email at Nichole.Korpi-Steiner@umassmemorial.org
- Genetic counselors are available for questions through client services 508-334-2863



Genomic Microarray Analysis



The UMass Memorial Medical Center Cytogenetics Laboratory is now offering Genomic Microarray Analysis to complement our extensive test menu. Conventional cytogenetic analysis is limited in its ability to detect and/or characterize chromosome abnormalities of less than approximately 5-10 Mb in size, as well as larger changes that do not alter the standard G-banding karyotype pattern. Genomic microarray analysis can detect chromosome imbalances up to 10 times smaller than those found by conventional cytogenetics, as well as those that may be hidden by the limitations of G-banding. This test utilizes comparative genomic hybridization (CGH) with over 43,000 oligonucleotide probes to identify chromosomal imbalances. The array provides high-density probe coverage of all known microdeletion/microduplication syndrome regions, as well as both the subtelomeric and pericentromeric regions of the chromosomes. In addition, the UMass array has probes spaced at an average of 75 kb across the genome to identify duplications and/or deletions of ~500 kb or greater.

Utility of Microarray Testing:

- For patients who have had a normal chromosome analysis but have an abnormal phenotype such as dysmorphic features or mental retardation, the analysis looks for imbalances smaller than the resolution of a standard karyotype.
- For patients with features that overlap more than one known microdeletion/microduplication syndrome, the analysis targets and tests all such regions simultaneously, providing complete results after a single analysis.
- For patients with a cytogenetically detected unbalanced chromosome abnormality (such as a translocation or marker chromosome), the analysis can precisely identify the unbalanced region(s) and define the size of the imbalance so that the involved genes may be examined.

- For patients with an “apparently balanced” chromosomal rearrangement and an abnormal phenotype, the analysis tests for possible cryptic deletions/duplications at the chromosomal breakpoints and across the genome.

Ordering information:

- Standard Chromosome analysis should be considered prior to or concurrent with microarray analysis.
- Consultation with a Clinical Geneticist is recommended.
- Specimen requirements: Whole blood in a green top (Sodium Heparin) tube. Adults: 10 ml; Children: 5 ml; Infants: 3 ml.
- Send sample at room temperature or refrigerated. Do not freeze sample.
- Microarray analysis may be ordered on the UMass Memorial Cytogenetics Requisition and must be accompanied by a signed consent form or consent verification (at the bottom of the Cytogenetic Requisition).

Methodology:

- DNA is isolated from a peripheral blood sample. The patient DNA is then fluorescently labeled and mixed in equal proportion with same sex control DNA labeled with a different fluorescent dye.
- The combined DNAs are hybridized to the microarray slide containing the oligonucleotide probes, and relative concentrations of patient DNA and control DNA at each probe site are compared to determine if there is a loss or gain of material.
- The UMass oligonucleotide microarray chip is based on the design of the EmArray Cyto6000, developed at Emory Genetics Laboratory (Atlanta, GA) as part of the International Standard Cytogenomic Array Consortium (ISCA), and is manufactured by Agilent Technologies Inc.

Data Interpretation:

- FISH analysis or conventional cytogenetics is used to confirm any abnormality detected via microarray. When an abnormal microarray result is obtained, parental peripheral blood specimens may be requested for FISH or microarray analysis to aid in interpretation and/or determine recurrence risks.
- All results are interpreted by ABMG Certified Cytogeneticists and described using standard ISCN 2009 array CGH nomenclature. A written report containing a summary and interpretation of the microarray findings is also provided.

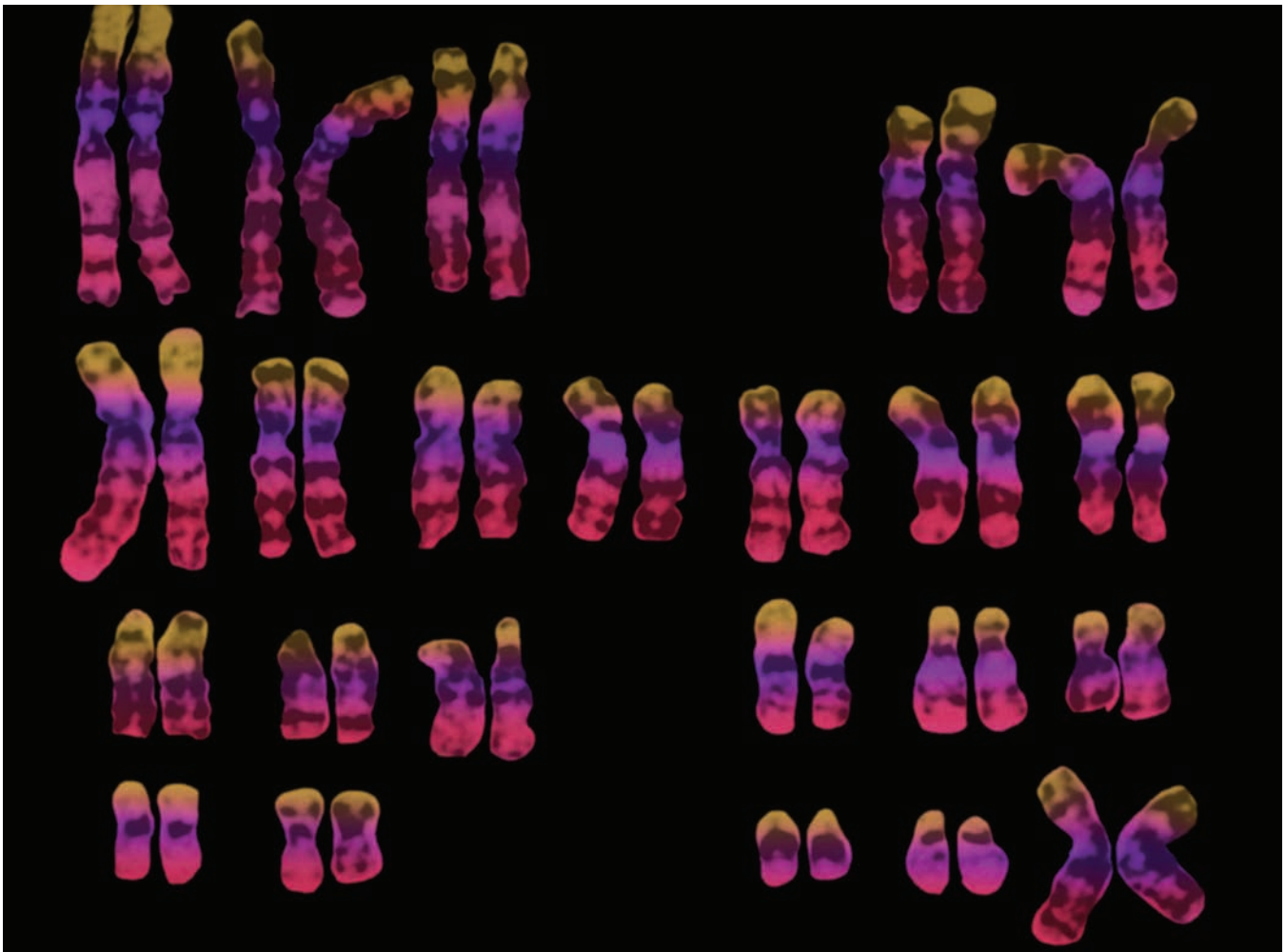
Limitations:

- Microarray analysis will not detect balanced chromosomal rearrangements such as translocations or inversions.
- Microarray testing can not detect point mutations, copy number changes in areas of the chromosomes that are not represented by the oligonucleotide array (that is, in areas between probes), or low-level mosaicism.
- This technique detects imbalances that are relatively small by cytogenetic standards. However, duplications or deletions smaller than 500 kb will not be investigated or reported unless they encompass one of the genomic locations specifically targeted on this array.

References:

1. Baldwin, Erin L, et al. "Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray." *Genet Med* 10(2008):415-429.
2. Lee, C et al. "Copy number variation (CNV) and clinical cytogenetic diagnostics." *Nat Genet* 39(2007): S49- S54
3. Mantripragada, Kiran K, et al. "Genomic microarrays in the spotlight." *Trends Genet* 20(2004): 87-94.

Please contact the UMass Memorial Cytogenetics Laboratory at 508-334-2863 if you have any questions regarding this testing.



Genomic Microarray Targeted Regions

Condition	MIM#	Gene(s)	Locus
All unique telomeric regions			41 sites
All unique centromeric regions			43 sites
Aneuploidy for all 24 chromosomes			24 chromosomes
15q11 BP1-BP2 interval		NIPA1 /NIPA2	15q11.2
15q26.3 deletion with IUGR; duplication with overgrowth	147370	IGF1R	15q26.3
17q21 deletion syndrome	610443	MAPT	17q21.31
1p telomere deletion syndrome	607872		1p36
22q telomere deletion syndrome	606232	SHANK3	22q13.33
22q11.2 deletion syndrome	188400, 192430	HIRA /TBX1	22q11.21
3q telomere deletion syndrome	609425		3q29
8p23.1 duplication/deletion syndrome	600576	GATA4	8p23.1
9q telomere deletion syndrome	610253	EHMT1	9q34.3
Alagille syndrome	118450	JAG1	20p12.2
Alpha-thalassemia/MR	301040	ATRX	Xq21.1
Alpha-thalassemia/MR syndrome	141750	HBA1 /HBA2	16p13.3
Alport syndrome	301050	COL4A5	Xq22.3
Androgen insensitivity syndrome	300068	AR	Xq12
Angelman syndrome	105830	UBE3A	15q11.2
Aniridia, type II	106210	PAX6	11p13
Autistic Features, X-linked	300495	NLGN4	Xp22.32-p22.31
Bannayan-Riley-Ruvalcaba syndrome	153480	PTEN	10q23.31
Basal cell nevus/Gorlin-Goltz syndrome	109400	PTCH	9q22.32
Beckwith-Wiedemann syndrome	130650	IGF2 /CDKN1C /KCNQ1 /H19	11p15.5
Blepharophimosis, ptosis, and epicanthus inversus (BPES)	110100	FOXL2	3q22.3
Brachydactyly, type C (BDC)	113100	GDF5	20q11.22
Brachydactyly-MR syndrome 600430 2q37.3			
Branchio-Oto-Renal syndrome/ Oto-Facio-Cervical syndrome	113650,166780	EYA1	8q13.3
Campomelic dysplasia (CMPD)	114290	SOX9	17q24.3
Cat-eye syndrome	115470		22q11.1
Charcot-Marie-Tooth disease type 1A	118220	PMP22	17p12
CHARGE syndrome	214800	CHD7	8q12.2
Cleft palate	119540	SATB2	2q33.1
Cleidocranial dysplasia	119600	RUNX2	6p21.1

Alphabetical (Point mutations, balanced, or small rearrangements, including small copy number changes within targeted regions, may not be detected.)

Genomic Microarray Targeted Regions

Condition	MIM#	Gene(s)	Locus
COL1A1 associated connective tissue disorders	120150	COL1A1	17q21.33
COL1A2 associated connective tissue disorders	120160	COL1A2	7q21.3
COL2A1 associated connective tissue and skeletal disorders	120140	COL2A1	12q13.11
Congenital adrenal hypoplasia/ Dosage sensitive sex reversal	300200, 300018	NR0B1 (DAX1)	Xp21.2
Congenital diaphragmatic hernia	142340	NR2F2 /CHD2	15q26.2
Cornelia de Lange syndrome	122470	NIPBL	5p13.2
Cornelia de Lange syndrome (CDLS), X-linked	300590	SMC1L1 (SMC1A)	Xp11.22
Cowden syndrome	158350	PTEN	10q23.31
Craniosynostosis with 11p15.2 disruption	607257	SOX6	11p15.2-p15.1
Cri-du-Chat syndrome	123450		5p15.2
Dandy-Walker malformation	220200	ZIC1 /ZIC4	3q24
DiGeorge syndrome 2	601362		10p14
Duchenne muscular dystrophy	310200	DMD	Xp21.2
Duplication 17p11.2 syndrome	610883		17p11.2
Familial adenomatous polyposis/ Gardner/Mental Retardation	175100	APC	5q22.2
Feingold syndrome	164280	MYCN	2p24.3
Fragile X mental retardation syndrome	300624	FMR1	Xq27.3
Glycerol kinase deficiency	307030	GK	Xp21.2
Greig cephalopolysyndactyly syndrome	175700	GLI3	7p14.1
Hemophilia A	306700	F8	Xq28
Hereditary neuropathy with liability to pressure palsies	162500	PMP22	17p12
Holoprosencephaly 1	236100	TMEM1	21q22.3
Holoprosencephaly 2	157170	SIX3	2p21
Holoprosencephaly 3	142945	SHH	7q36.3
Holoprosencephaly 4	142946	TGIF	18p11.31
Holoprosencephaly 5	609637	ZIC2	13q32.3
Holoprosencephaly 6	605934		2q37.1-q37.3
Holoprosencephaly 7	610828	PTCH	9q22.32
Holoprosencephaly 9	165230	GLI2	2q14.2
Hypogammaglobulinemia, isolated growth hormone deficiency	307200	BTK	Xq22.1
Hypoparathyroidism, sensorineural deafness, and renal disease	146255	GATA3	10p14
Incontinentia pigmenti	308300	IKBKG	Xq28

Genomic Microarray Targeted Regions

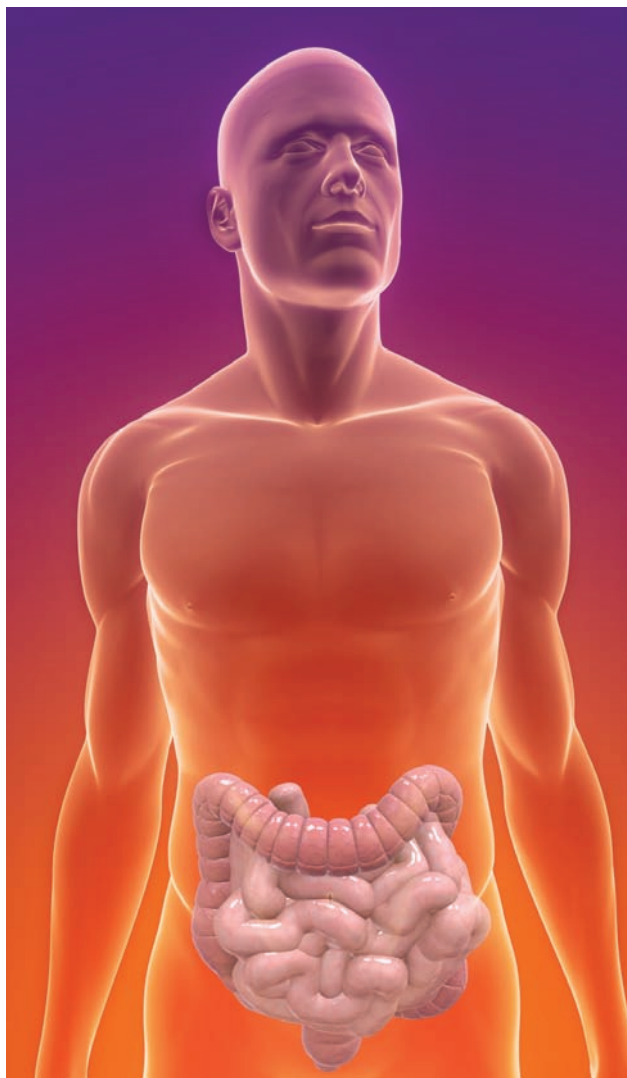
Condition	MIM#	Gene(s)	Locus
Infantile spasm syndrome, X-linked	308350	ARX	Xp21.3
Infantile spasms with CDKL5 deficiency	300203	CDKL5	Xp22.13
Jacobsen syndrome/11q terminal deletion disorder	147791		11q25
Joubert syndrome 4	609583	NPHP1	2q13
Juvenile Nephronophthisis	256100	NPHP1	2q13
Kallmann syndrome 1	308700	KAL1	Xp22.31
Langer-Giedion syndrome	150230	EXT1 /TRPS1	8q24.11
Leri-Weill dyschondrosteosis	127300	SHOX	Xp22.33/Yp11.32
Lesch-Nyhan syndrome	300322	HPRT1	Xq26.2
Loeys-Dietz syndrome	609192	TGFBR2	3p24.1
Loeys-Dietz syndrome	609192	TGFBR1	9q22.33
Marfan syndrome	154700	FBN1	15q21.1
Marfan syndrome type II	154705	TGFBR2	3p24.1
Menkes disease	309400	ATP7A	Xq21.1
Microphthalmia, syndromic 3	206900	SOX2	3q26.33
Microphthalmia, syndromic 7	309801	HCCS	Xp22.2
Miller-Dieker/Lisencephaly	247200	PAFAH1B1 (LIS1)	17p13.3
Mitochondrial Complex I deficiency	161015	NDUFV1	11q13.2
Mowat-Wilson syndrome	235730	ZEB2	2q22.3
MR, X-linked 21 (MRX21)	300143	IL1RAPL1	Xp21.3-p21.2
Mucopolysaccharidosis, type II (Hunter syndrome)	309900	IDS	Xq28
Nail-Patella Syndrome	161200	LMX1B	9q33.3
Neurofibromatosis 1	162200	NF1	17q11.2
Neurofibromatosis 2	101000	NF2	22q12.2
Noonan syndrome 1	163950	PTPN11	12q24.13
Opitz syndrome	300000	MID1	Xp22.2
Ornithine transcarbamylase deficiency	311250	OTC	Xp11.4
Orofaciodigital syndrome	311200	OFD1	Xp22.2
Pallister-Killian syndrome	601803		12p
Pelizaeus-Merzbacher disease	312080	PLP1	Xq22.2
Polycystic kidney disease	601313	PKD1	16p13.3
Potocki-Shaffer syndrome	601224	EXT2 /ALX4	11p11.2
Prader-Willi syndrome	176270	SNRPN	15q11.2
Prader-Willi-like phenotype	603128, 176270	SIM1	6q16.3
Renal cysts and diabetes syndrome	137920	TCF2	17q12
Retinoblastoma	180200	RB1	13q14.2

Genomic Microarray Targeted Regions

Condition	MIM#	Gene(s)	Locus
Retinoschisis, X-linked juvenile	312700	RS1	Xp22.13
Rett syndrome	312750	MECP2	Xq28
Rieger syndrome, type 1	180500	PITX2	4q25
Rubinstein-Taybi syndrome	180849	CREBBP	16p13.3
Saethre-Chotzen syndrome	101400	TWIST1	7p21.1
Sex Determining Region Y	480000	SRY	Yp11.31
Smith-Magenis syndrome	182290	RAI1	17p11.2
Sotos syndrome	117550	NSD1	5q35.3
Speech-Language Disorder 1	602081	FOXP2	7q31.1
Split-Hand/Foot Malformation 1	183600	SHFM1	7q21.3
Split-Hand/Foot Malformation 3	600095	FBXW4	10q24.32
Split-Hand/Foot Malformation 4	605289	TP73L	3q28
Split-Hand/Foot Malformation 5	606708		2q31.1
Steroid sulfatase deficiency	308100	STS	Xp22.31
Stickler syndrome, type II	604841	COL11A1	1p21.1
Synpolydactyly I/Syndactyly II	186000	HOXD gene cluster	2q31.1
Treacher Collins syndrome	154500	TCOF1	5q32
Trichorhinophalangeal syndrome, type1	190350	TRPS1	8q23.3
Tuberous sclerosis 1	191100	TSC1	9q34.13
Tuberous sclerosis 2	191100	TSC2	16p13.3
Van der Woude syndrome	119300	IRF6	1q32.2
Waardenburg syndrome, type IIA	193510	MITF	3p13
WAGR syndrome	194072	PAX6 /WT1	11p13
Williams syndrome	194050	ELN /LIMK1	7q11.23
Wilms Tumor	194070	WT1	11p13
Wolf-Hirschhorn syndrome	194190	WHSC1 /WHSC2	4p16.3
X Inactivation Center	314670	XIST	Xq13.2
X-linked heterotaxy	306955	ZIC3	Xq26.3
X-linked lissencephaly	300067	DCX	Xq23
X-linked lymphoproliferative syndrome	308240	SH2D1A /BIRC4	Xq25
X-linked mental retardation with isolated growth hormone deficiency	300123	SOX3	Xq27.1
X-linked MR with retinitis pigmentosa	300578	RP2	Xp11.3
XY Sex-reversal +/- adrenal failure	184757	NR5A1	9q33.3

Reference: Baldwin et al. "Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray." *Genet Med*, 2008.

Changes in Laboratory Testing for Celiac Disease



Celiac disease (also known as gluten-sensitive enteropathy or nontropical sprue) is an immune-mediated inflammation of the small intestine caused by sensitivity to dietary gluten and related proteins in genetically sensitive individuals. The disorder is common, occurring in 0.5 to 1 percent of the general population. It is now considered to be among the most common, undiagnosed chronic diseases. The grains that contain the triggering proteins are wheat, barley, and rye; there is some controversy as to whether oats also can cause the disease. The small intestinal mucosa improves morphologically when treated with a gluten-free diet and relapses when gluten is reintroduced.

Intestinal biopsy remains the gold standard for definitive diagnosis of celiac disease. However, serological testing has been suggested for screening patients with suspected gluten

sensitive enteropathy as well as for monitoring dietary compliance. A variety of serologic studies have been described to aid in the diagnosis of celiac disease. They can be divided into two groups based upon their target antigens: antiendomysial and antigliadin antibody tests

Endomysial antibodies bind to connective tissue surrounding smooth muscle cells. Sections of monkey esophagus or sections of human umbilical cord are used by different laboratories. Serum IgA endomysial antibodies bind to the endomysium, producing a characteristic staining pattern, which is visualized by indirect immunofluorescence. This type of screening requires highly skilled and trained eye and somewhat subjective depending on the ability of technologist in looking at a staining pattern. This test is rarely performed now.

The antigen against which antiendomysial antibodies are directed is a tissue transglutaminase (tTG). tTG is the major target antigen recognized by anti-endomysium antibodies. Anti-tTG antibodies are highly sensitive and specific for the diagnosis of celiac disease. Studies confirmed that, anti-tTG antibodies are present in 98 percent of patients with biopsy-proven celiac disease compared to 5 percent of controls. ELISA tests for IgA anti-tTG antibodies are widely available and are easier to perform and less costly than the immunofluorescence assay used to detect IgA endomysial antibodies.

Gliadin is a component of the wheat storage protein gluten. Serum antigliadin antibody levels are frequently elevated in untreated celiac disease, and antigliadin assays have been used for some years as a diagnostic aid. It is thought that food derived gliadin peptides are selectively deamidated (the conversion of the amino acid glutamine to glutamic acid) in the gut by the enzyme tTG. This selective deamidation of gliadin peptides may be the event that initiates the immune response to gluten and then tTG in genetically predisposed individuals. Newly developed ELISA-based deamidated gliadin (DGP) antibody assays are more useful in the diagnosis of celiac disease than the native gliadin antibody assays. DGP assays seems to be equivalent to, but not better than, tTG-IgA. However, it may have additive benefits in celiac screening because the combination of the 2 tests can increase the sensitivity without actually lowering the specificity. The DGP test also may be beneficial in circumstances when the tTG results are indeterminate. Also, among young children it seems to appear before tTG and to resolve faster in the context of gluten withdrawal.

Testing for celiac disease in the general population is not recommended.

Testing should be considered in the following groups of patients:

- Those with gastrointestinal symptoms including chronic or recurrent diarrhea, malabsorption, weight loss, and abdominal distension or bloating. This includes patients with symptoms suggestive for irritable bowel syndrome or severe lactose intolerance.
- Individuals without other explanations for signs and symptoms such as iron deficiency anemia, folate or vitamin B12 deficiency, persistent elevation in serum aminotransferases, short stature, delayed puberty, recurrent fetal loss, low birth-weight infants, and reduced fertility persistent aphthous stomatitis, dental enamel hypoplasia, idiopathic peripheral neuropathy, nonhereditary cerebellar ataxia, or recurrent migraine headaches.
- Symptomatic individuals at high risk for celiac disease including patients with type 1 diabetes mellitus or other autoimmune disorders, first- and second-degree relatives of individuals with celiac disease, patients with Turner, Down, or Williams syndromes.

All testing should be performed while patients are on a gluten-rich diet. Some patients may have already begun a low gluten diet before undergoing formal evaluation and thus may have normal results from antibody testing. Such patients should be advised to consider resuming a gluten-rich diet for 2 to 12 weeks before antibody titers are drawn. No single test can confidently establish the diagnosis of celiac disease in every individual. As a result, the most important initial step in diagnosis is recognition of the many clinical features that can be associated with the disease.

IgA deficiency is more common in celiac disease (2 to 5 percent) than in the general population (<0.5 percent). The tTG-IgA serology tests will be falsely negative in untreated celiac disease in patients with IgA deficiency. As a result, total serum IgA can be measured in addition to tTG-IgA especially when there is heightened clinical suspicion for celiac disease and IgA markers are negative. If total IgA levels are abnormally low, an IgG-based tTG assay should be used to test for celiac disease. Thus, serum tTG-IgG or DGP-IgG tests are preferable. In addition to serologic markers, the diagnosis usually requires a small bowel biopsy, which can be obtained during upper endoscopy.

There are three main possibilities in those with suggestive clinical features but negative serologic tests:

- The individual may have selective IgA deficiency. In such patients, testing for tTG-IgG antibodies and/or DGP- IgG antibodies should be performed.
- The individual may already be on a low gluten diet.
- The serologic test could be falsely negative in which case a small bowel biopsy is needed to make a diagnosis.



- The patient may not have celiac disease in which case other causes of symptoms or villous atrophy should be considered.

There are occasional patients in whom the diagnosis is unclear despite the above. Such patients can undergo testing for HLA haplotypes associated with celiac disease. More than 99 percent of patients with celiac disease have HLA DQ2 and/or DQ8 compared with about 40 percent of the general population. Thus, celiac disease is highly unlikely in patients without these haplotypes.

The current recommendations for use of these tests are:

- For the vast majority of celiac screening and follow-up, tTG-IgA testing is strongly recommended.
- We recommend ordering both total serum IgA levels in conjunction with the initial tTG-IgA in every patient. IgA deficiency is more common among celiac patients than in the general population (i.e., more than 1 in 400). IgA deficiency will cause a falsely negative tTG-IgA. IgA levels are extremely cost-effective compared to other celiac serologic tests (e.g., anti-DGP), which should be ordered if IgA deficiency is established.

Consider using anti-DGP in the following exceptional situations:

- In cases of known IgA deficiency for initial evaluation for celiac disease
- In cases of known IgA deficiency with celiac disease to monitor response to dietary therapy
- When IgA anti-tTG is normal in patients with villous atrophy
- In patients with a high pre-test probability of celiac disease but a negative IgA anti-tTG to guide a decision regarding need for endoscopy and biopsy

Effective November 30, 2010, tTG-IgG, DGP-IGA and DGP-IGG serology testing will be performed in house. The test will be performed on serum samples and will be available Monday through Friday. There will be no changes in the test mnemonics, reference ranges or sample requirements.

Negative	<20 Units
Weak Positive	20 – 30 Units
Moderate Positive to Strong Positive	>30 Units

The test reports include a semi-quantitative value along with the test interpretation. The results of each antibody are expressed as antibody units.

The following comprehensive list of various celiac serology tests available:

Mnemonic	Test
CELEV	IGA Total, tTG-IGA (If IGA total <7 mg/dL automatic reflex to tTG-IgG)
CELREF	IGA Total, tTG-IGA (If IGA total <7 mg/dL automatic reflex to tTG-IgG and DGP-IgG)
CELCOMP	IGA Total, tTG-IGA, tTG-IgG, DGP-IgA and DGP-IgG)
TGLA	tTG-IGA
TRANSGL	tTG-IGG
AGA	DGP-IgA and DGP-IgG
AGIGA	DGP-IgA
AGIGG	DGP-IgG

For any questions, comments and suggestions, please contact:

- Dr. L.V. Rao, Director of Core Laboratories at 774-442-9615 or via email at Lokinendi.Rao@umassmemorial.org
- Dr. M. Rabie Al-Turkmani, Associate Director of Immunology & Immunoassay at 774-442-9663 or via email at MRabie.Alturkmani@umassmemorial.org
- Ms. Rachel Ambacher, Manager of Immunology & Immunoassay at 774-442-9065 or via email at Rachel.Ambacher@umassmemorial.org



Changes in Amylase Testing



Amylase is an enzyme that helps digest glycogen and starch. It is produced mainly in the pancreas and salivary glands. Amylase is normally secreted from the pancreas through the pancreatic duct into the small intestine. Elevated serum levels are associated with acute pancreatitis and other pancreatic disorders as well as mumps and bacterial parotitis. Amylase activity in serum tends to increase rapidly after an attack of pancreatitis and may be demonstrated as early as six to eight hours after its onset. Levels stay elevated for one to three days and then return rapidly to normal, reflecting the efficient renal clearance of the enzyme.

Decreased amylase levels have been found in abscesses of the liver, acute hepatocellular damage, cirrhosis, cancer of the liver and bile duct and cholecystitis.

Amylase is a relatively small protein and is therefore filtered readily into the urine. The enzyme can be found in increased concentrations in the urine for longer periods of time than in the serum. Urine Amylase is used in the differential diagnosis of pancreatitis. It is very useful in the diagnosis of pseudocyst of the pancreas, where the urine amylase may remain elevated for weeks after the serum amylase has returned to normal, and after a bout of acute pancreatitis.

Effective November 30, 2010, UMass clinical laboratories will perform Amylase testing in serum and Plasma by IFCC recommended enzymatic G7 method. This methodology will significantly improve the quality and accuracy of amylase testing in various disease conditions. There will be no changes in the test mnemonics or sample requirements.

Validation studies conducted both at UMass Labs and others observed there is a 20-25% lower bias compared to existing methodology. Based on this there will be a change in the reference range. The new reference range for serum will be 28-100 U/L. The new reference range for Urine 16-491 U/L (Males) and 21-447 U/L (Females).

For any questions, comments and suggestions please contact:

- Dr. L.V. Rao, Director of Core Laboratories at 774-442-9615 or via email at Lokinendi.Rao@umassmemorial.org
- Ms. Judy Barron, Manager of Automated Chemistry at 774-442-9616 or via email at Judy.Barron@umassmemorial.org

Changes in Platelet Aggregation Testing

Effective November 1, 2010, the following changes will be made to Platelet aggregation testing. We will now perform whole blood platelet aggregation which will include aggregation and ATP release. Specimen requirements and scheduling of this test will remain the same.

For any questions, comments and suggestions please contact:

- Dr. Hongbo Yu, at 774-442-9635 or via email at Hongbo.Yu@umassmemorial.org

Aggregation Agonist	Reagent Concentration	Normal Range	Release Agonist	Reagent Concentration	Normal Range
Collagen	5 ug/mL	> 15 ohms	Thrombin		> 0.5 nm
Collagen	1 ug/mL	> 15 ohms	Collagen	5 ug/mL	> 0.8 nm
Arachidonic Acid	0.5 mM	> 5 ohms	Collagen	1 ug/mL	> 0.5 nm
ADP	10 uM	> 5 ohms	Arachidonic Acid	0.5 mM	> 0.3 nm
Ristocetin	1.0 mg/mL	> 5 ohms	ADP	10 uM	> 0.2 nm
Ristocetin	0.25 mg/mL	0 ohms			



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This publication is made possible by Kevin Vance, Senior Director, Business Development and Marketing



Photo: Kevin Vance

UMass Memorial Laboratories operates three laboratories in Worcester, Massachusetts, including a regional laboratory that is located in 38,000 square feet of state-of-the-art lab space in the Biotech Park, as well as laboratories at the University campus and Memorial campus of UMass Memorial Medical Center.