TEXAS A&M TVMDL VETERINARY MEDICAL DIAGNOSTIC LABORATORY

FAQ: *G6S diagnostic testing*

Q: What is G6S, and why should I test for it?
Caprine Mucopolysaccharideosis-IIID is a lysosomal storage disorder, caused by a genetic mutation (a point mutation) which results in a defective G6S (N-Acetylglucosamine-6-sulfatase) enzyme. The only method for testing for this genetic defect is with a DNA test identifying the causative point mutation.

Goats should be tested prior to breeding to minimize the risk of future kids born with this heritable defect. G6S deficient goats may present with neurological deficiencies, which negatively affects growth and development, and can often lead to early death.

Q: When can G6S testing be done?
G6S is a genetic defect. As such, a goat may be tested at any stage of life, from birth to old age.

Q: What does a "Normal," "Carrier," or "Affected" result mean?
A Normal result means that the goat does not possess the causative genetic mutation on either of the two alleles (i.e. possesses two copies of the normal G6S gene), and therefore does not have the G6S deficiency. Breeding of these individuals' results in genotypically (genetically) and phenotypically (physical appearance) normal offspring.

A Carrier result means that the goat carries one copy of the causative mutation, which was inherited from one of the two parents (i.e., the mutation is present on one of the two inherited alleles). Therefore, a carrier will possess one normal allele, and also carry one mutated allele (i.e., one copy of the normal gene and one copy of the defective gene). Carrier animals will appear normal since the disorder only manifests under a recessive mode of inheritance, (i.e., two mutated or defective alleles for the genetic defect to manifest clinical disease). Importantly, carriers can pass one mutant (defective) allele on to their offspring. For example: If one parent possesses one copy of the recessive (defective) allele (a), there is a 50% chance of passing it to their offspring. Thus, genotypically, 50% of the offspring are expected to possess the recessive (mutant) allele; however, phenotypically, 100% will appear normal and be unaffected.

	A	A
A	AA(25%)	AA(25%)
a	Aa(25%)	Aa(25%)

An additional example: two phenotypically normal goats can both be genotypically carriers (Aa), and thus if they are mated then the following can occur:

	A	a
A	AA(25%)	Aa(25%)
a	Aa(25%)	aa(25%)

In this case, there is a 75% chance of being phenotypically normal (AA, Aa x 2) and a 25% chance of being genotypically normal (ie. AA, non-carrier). Moreover, there is a 25% chance that the offspring of two carriers will inherit the genotype most commonly associated with the G6S deficiency (ie. aa, affected).
It is important to note that this probability is reset for each instance of mating/breeding. Each offspring has the same probability.

An Affected diagnostic result should be interpreted to mean that the individual possesses two mutantalleles (aa) (i.e., the animal has two copies of the defective gene), and therefore, the goat has the genotype associated with the G6S deficiency.

Q: What is the prevalence of this mutation?
In the last decade, researchers estimate that the overall prevalence of this defect in the Nubian goat population is approximately: 74.2% Normal, 23.9% Carrier, and 1.9% Affected individuals.

Q: How can two Normal parents create a Carrier when breeding stock was tested years ago?
No diagnostic test is perfect; all such tests have varying degrees of error. As technology improves error rates are reduced, but will not be completely eliminated (i.e., zero error).

Until 2003, the test method utilized for identification of the G6S mutation was the best technology available at the time, which was a gel based genotyping assay. However, this test method can potentially produce genotyping error rates of 2% to 5%, meaning that 2-to-5 out of every 100 animals tested could potentially be misidentified as genetically normal, but may have in fact been carriers. In 2003, a more sensitive and specific assay was implemented using a newer technology (i.e., a probe based real-time PCR assay), which resulted in a reduced error rate (i.e., approximately 1%); meaning that 1 out of 100 animals tested could potentially be misidentified.

Below is a table of test results generated from 2000 to 2013 at TVMDL:

Year	Affected		Carrier		Normal	
2000	0/78	0.00%	18/78	23.08%	60/78	76.92%
2001	3/235	1.28%	41/235	17.45%	191/235	81.28%
2002	1/176	0.57%	24/176	13.64%	151/176	85.80%
2003	2/58	3.45%	16/58	27.59%	40/58	68.97%
2003[a]	0/123	0.00%	41/123	33.33%	82/123	66.67%
2004	1/92	1.09%	24/92	26.09%	67/92	72.83%
2005	7/171	4.09%	38/171	22.22%	124/171	72.51%
2006	0/120	0.00%	30/120	25.00%	90/120	75.00%
2007	3/150	2.00%	36/150	24.00%	111/150	74.00%
2008	2/97	2.06%	24/97	24.74%	71/97	73.20%
2009	5/235	2.13%	44/235	18.72%	186/235	79.15%
2010	1/347	0.29%	59/347	17.00%	287/347	82.71%
2011	2/370	0.54%	76/370	20.54%	292/370	78.92%
2012	1/384	0.26%	76/384	19.53%	308/384	80.21%
2013	5/586	0.85%	110/586	18.77%	471/586	80.38%
Total	**33/3225**	**1.02%**	**656/3225**	**20.34%**	**2534/3225**	**78.57%**

[a]Implementation of newer technology for testing.

Q: If the prevalence hasn't changed over the years, why continue to test?
Knowing the genetic status of a breeding pair will assist in making breeding decisions which will greatly reduce the probability of producing affected individuals. Carrier animals can live long, productive lives but their presence in the breeding population should be managed accordingly.

Q: How frequently should I test?
Molecular methodologies are continually advancing; therefore, improved test methods will be implemented as they become available. Even with the advancement in testing methods, error rates continue to exist. In order to reduce the error rate, every animal in each generation should be tested.

Q: What types of samples are best for G6S testing?
1-2 mL (or cc) of EDTA whole blood (in purple top tubes) is ideal for G6S testing. Using the purple top, EDTA coated tubes for collection prevents the blood sample from clotting. Clotted blood is not recommended for G6S testing. At this time, we are not testing semen samples for G6S.

Q: How do I submit samples for G6S testing?
Samples should be shipped overnight whenever possible. They should be appropriately packed prior to shipping. Please refer to our shipping information page for details: http://tvmdl.tamu.edu/shipping/how-to-ship-a-sample-to-tvmdl/

Q: Where can I find more information about G6S?
1. Clavijo, A., F. Sun, L. Sneed (2010) Diagnosis of caprine mucopolysaccharidosis type IIID by real-time polymerase chain reaction-based genotyping. *Journal of Veterinary Diagnostic Investigation* 22:622-627.

2. Hoard, H.M. et al. (1998) Determination of genotypic frequency of caprine mucopolysaccharidosis IIID. *Journal of Veterinary Diagnostic Investigation* 10: 181-183.

Q: Does TVMDL hold a patent on the G6S PCR test?
No. The assay (test) details are published publically in the peer reviewed Journal of Veterinary Diagnostic Investigation and TVMDL holds no patent on the test. This test was updated to newer technology (qPCR) from its original gel-based PCR, as described in the referenced article, and is offered by TVMDL as a service to the caprine industry.

For more information, read Clavijo, A., F. Sun, L. Sneed (2010) Diagnosis of caprine mucopolysaccharidosis type IIID by real-time polymerase chain reaction-based genotyping. *Journal of Veterinary Diagnostic Investigation* 22:622-627.

Q: Does re-validating mean TVMDL's past test results based on semen are invalid?
Results from testing semen are as valid as the results from testing blood. Periodically, all PCR assays should undergo a revalidation process to make sure the test is performing as expected. With this particular situation, extraction methods have changed from the time the assay was published and TVMDL would like to ensure the performance of the G6S PCR for semen.

TVMDL welcomes questions on our G6S testing. Contact the agency headquarters at 1.888.646.5623.