

**Third National Health and Nutrition  
Examination Survey 1988–1994**

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**Documentation, Codebook,  
and Frequencies**

**Inhibin B, Luteinizing hormone,  
Testosterone**

**Laboratory  
Surplus Sera**

**Survey Years:  
1988 to 1994**

**SAS Export File:  
SSNH3HRM.XPT**



**First Published: June 2009  
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# NHANES III Data Documentation

## Laboratory Assessment: Inhibin B, Luteinizing hormone, Testosterone

(Participants Ages 6-11, Surplus Sera 1988 -1994)

Years of Coverage: 1988-1994

First Published: June 2009 Last Revised: December 2009

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### Component Description

Measurement of inhibin B, luteinizing hormone (LH), and testosterone from stored sera specimens.

### Eligible Sample

Participants 6 - 11 years of age from NHANES III, 1988-1994 with stored sera. Inhibin B, LH and testosterone were measured for boys; inhibin B and LH for girls.

### Description of Laboratory Methodology

Inhibin B Assay: The DSL-10-84100 ACTIVE® Inhibin B ELISA is an enzymatically amplified two-site two-step sandwich-type immunoassay. Standards, controls and unknown serum samples were incubated in microtitration wells, coated with anti-inhibin  $\beta_B$  subunit antibody. After incubation and washing, the wells were incubated with biotinylated anti-inhibin  $\alpha$ -subunit detection antibody and the immunoreaction monitored by subsequent addition of streptavidin labeled with enzyme horseradish peroxidase (HRP). After a third incubation and washing step, the wells were incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured is directly proportional to the concentration of inhibin B present. Inhibin B concentration was calculated from the standard curve.

The assay performance characteristics are as follows:

Intra-assay precision (serum):

Mean = 69 pg/ml	CV = 3.5%	n=23
Mean = 274 pg/ml	CV = 4.6%	n=23
Mean = 472 pg/ml	CV = 5.6%	n=23

Inter-assay precision (serum):

Mean= 50.1 pg/ml	CV = 7.6%	n=14
Mean= 188.4 pg/ml	CV = 6.3%	n=14
Mean= 355.0 pg/ml	CV = 6.2%	n=14

Functional sensitivity (serum): 7 pg/ml (range: 5.9-251 pg/ml)

Kit source: Diagnostic Systems Laboratories, Inc.

<http://www.dslabs.com>

Luteinizing Hormone (LH) Assay: The LH ELISA kit (Bio-Quant BQ049F) is a solid phase direct sandwich method. The samples and diluted anti-LH-HRP conjugate were added to the wells coated with MAb to LH beta subunit. During a 30-minute incubation at room temperature, the LH in serum binds to anti-LH MAb on the well and the anti-LH second antibody then binds to LH. Unbound protein and HRP conjugate were washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of LH in the samples. A standard curve was prepared relating color intensity to the concentration of the LH. The test kit is designed for in-vitro diagnostic use. The Bio-Rad Lyphocheck Fertility control was run on each plate as a positive control.

The assay performance characteristics are as follows:

Intra-assay precision (serum):

Mean = 1.7 mIU/ml	CV = 10.58%	n=16
Mean = 16.3 mIU/ml	CV = 7.60%	n=16
Mean = 47.2 mIU/ml	CV = 6.18%	n=16

Inter-assay precision (serum):

Mean = 1.9 mIU/ml	CV = 11.57%	n=10
Mean = 15.6 mIU/ml	CV = 10.83%	n=10
Mean = 46.1 mIU/ml	CV = 8.13%	n=10

Functional sensitivity (serum): 0.05 mIU/ml (range: 2.4-57 mIU/ml)

Kit source: Bio-Quant, Inc.

<http://www.bio-quant.com>

Testosterone Assay: The Testosterone EIA (Bio-Quant BQ080S) was based on the principle of competitive binding between testosterone in the test specimen and testosterone-HRP conjugate for a constant amount of rabbit anti-testosterone. In the incubation, goat anti-rabbit IgG-coated wells were incubated with 10 µl of Testosterone standards, controls, patient samples, 100 µl testosterone-HRP conjugate reagent and 50 µl rabbit anti-testosterone reagent at 37°C for 90 minutes. During the incubation, a fixed amount of HRP-labeled testosterone competes with the endogenous testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific testosterone antibody. Thus, the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of testosterone in the specimen increases.

Unbound testosterone peroxidase conjugate was then removed and the wells washed. Next, a solution of TMB Reagent was added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development was stopped and the absorbance was measured. The intensity of the color formed is proportional to the amount of enzyme present and inversely related to the amount of unlabeled testosterone in the sample. The test kit is designed for *in vitro* diagnostic use. The Bio-Rad Lyphocheck Fertility control was be run on each plate as a positive control.

The assay performance characteristics are as follows:

Intra-assay precision (serum):

Mean = 3.7 ng/ml	CV = 10.0%	n=24
Mean = 5.1 ng/ml	CV = 8.3%	n=24
Mean = 12.7 ng/ml	CV = 5.0%	n=24

Inter-assay precision (serum):

Mean = 3.4 ng/ml	CV = 8.4%	n=20
Mean = 5.0 ng/ml	CV = 4.4%	n=20
Mean = 13.3 ng/ml	CV = 3.7%	n=20

Functional sensitivity (serum): 0.05 ng/ml (range: 0.1-10 ng/ml)

Kit source: Bio-Quant, Inc.

<http://www.bio-quant.com>

## Laboratory Quality Control and Monitoring

See description above.

## Data Processing and Editing

Data was received after all the laboratory testing was complete. Values that fell below the standard curve were set to values representing the minimum detectable concentration divided by the square root of 2 ( LOD /  $\sqrt{2}$  ):

Inhibin B	4.95 pg/ml	(LOD = 7 pg/ml)
Luteinizing Hormone	0.035 mIU/ml	(LOD = 0.05mIU/ml)
Testosterone	0.035 ng/ml	(LOD = 0.05 ng/ml)

Samples where the quantity was not sufficient (QNS) for analysis were set to missing. The data were not otherwise edited.

Data Access: All data are publicly available.

## Analytic Notes

There are 7 variables:

Sequence Number

InhibinB1, InhibinB2 (duplicates on same sample)

Luteinizing Hormone1, Luteinizing Hormone 2 (duplicates on same sample)

Testosterone1, Testosterone2 (duplicates on same sample)

## References

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**National Health and Nutrition Examination Survey  
Codebook for Data Production (1988-1994)  
(NHANES III)**

**Inhibin B, Leuteinizing hormone and Testosterone (SSNH3HRM)  
Person Level Data**

December 2009



<b>SEQN</b>	<b>Target</b>
	B(6 Yrs. to 11 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	Respondent sequence number
<b>English Text:</b> Respondent sequence number.	
<b>English Instructions:</b>	

<b>SSINHIB1</b>	<b>Target</b>
	B(6 Yrs. to 11 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	inhibin B 1
<b>English Text:</b> inhibin B 1	
<b>English Instructions:</b>	

Code or Value	Description	Count	Cumulative	Skip to Item
4.95 to 394	Range of Values	1510	1510	
.	Missing	41	1551	

<b>SSINHIB2</b>	<b>Target</b>
	B(6 Yrs. to 11 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	inhibin B 2
<b>English Text:</b> inhibin B 2	
<b>English Instructions:</b>	

Code or Value	Description	Count	Cumulative	Skip to Item
4.95 to 379	Range of Values	1391	1391	
.	Missing	160	1551	

<b>SSLH1</b>	<b>Target</b>
	B(6 Yrs. to 11 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	luteinizing hormone 1
<b>English Text:</b> luteinizing hormone 1	
<b>English Instructions:</b>	

Code or Value	Description	Count	Cumulative	Skip to Item
0.035 to 26.6	Range of Values	1545	1545	
.	Missing	6	1551	

<b>SSLH2</b>	<b>Target</b>
	B(6 Yrs. to 11 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	luteinizing hormone 2
<b>English Text:</b> luteinizing hormone 2	
<b>English Instructions:</b>	

Code or Value	Description	Count	Cumulative	Skip to Item
0.035 to 24.3	Range of Values	1445	1445	
.	Missing	106	1551	



<b>SSTEST1</b>		<b>Target</b>		
		B(6 Yrs. to 11 Yrs.)		
<b>Hard Edits</b>		<b>SAS Label</b>		
		testosterone 1		
<b>English Text:</b> testosterone 1				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
0.035 to 6.19	Range of Values	828	828	
.	Missing	723	1551	

<b>SSTEST2</b>		<b>Target</b>		
		B(6 Yrs. to 11 Yrs.)		
<b>Hard Edits</b>		<b>SAS Label</b>		
		testosterone 2		
<b>English Text:</b> testosterone 2				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
0.035 to 8.9	Range of Values	791	791	
.	Missing	760	1551	