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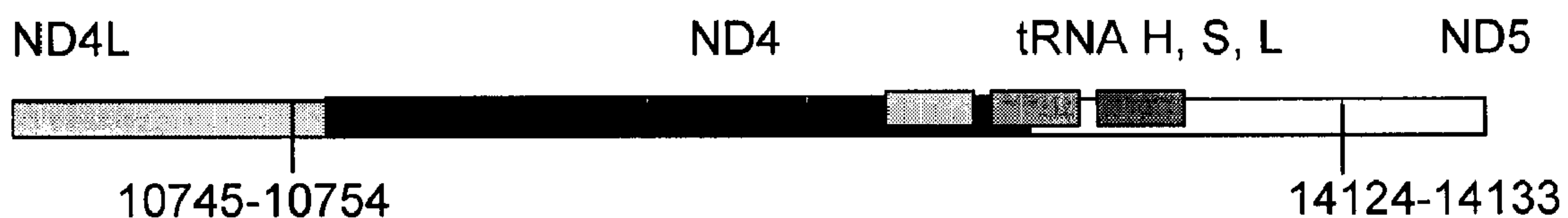
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(54) Title: ABERRANT MITOCHONDRIAL DNA, ASSOCIATED FUSION TRANSCRIPTS AND HYBRIDIZATION PROBES
THEREFOR



(57) Abrégé/Abstract:

The present invention provides novel mitochondrial fusion transcripts and the parent mutated mtDNA molecules that are useful for predicting, diagnosing and/or monitoring cancer. Hybridization probes complementary thereto for use in the methods of the invention are also provided.

1 **ABSTRACT**

2 The present invention provides novel mitochondrial fusion transcripts and the parent
3 mutated mtDNA molecules that are useful for predicting, diagnosing and/or monitoring cancer.
4 Hybridization probes complementary thereto for use in the methods of the invention are also
5 provided.

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**ABERRANT MITOCHONDRIAL DNA, ASSOCIATED FUSION TRANSCRIPTS AND
HYBRIDIZATION PROBES THEREFOR**

FIELD OF THE INVENTION

[0001] The present invention relates to the field of mitochondrial genomics. In one aspect, the invention relates to the identification and use of mitochondrial genome fusion transcripts and probes that hybridize thereto.

BACKGROUND OF THE INVENTION

[0002] Mitochondrial Genome

[0003] The mitochondrial genome is a compact yet critical sequence of nucleic acids. Mitochondrial DNA, or "mtDNA", comprises a small genome of 16,569 nucleic acid base pairs (bp) (Anderson et al., 1981; Andrews et al., 1999) in contrast to the immense nuclear genome of 3.3 billion bp (haploid). Its genetic complement is substantially smaller than that of its nuclear cell mate (0.0005%). However, individual cells carry anywhere from 10³ to 10⁴ mitochondria depending on specific cellular functions (Singh and Modica-Napolitano 2002). Communication or chemical signalling routinely occurs between the nuclear and mitochondrial genomes (Sherratt et al., 1997). Moreover, specific nuclear components are responsible for the maintenance and integrity of mitochondrial sequences (Croteau et al., 1999). All mtDNA genomes in a given individual are identical due to the clonal expansion of mitochondria within the ovum, once fertilization has occurred. However mutagenic events can induce sequence diversity reflected as somatic mutations. These mutations may accumulate in different tissues throughout the body in a condition known as heteroplasmy.

[0004] Mitochondrial Proteome

[0005] About 3,000 nuclear genes are required to construct, operate and maintain mitochondria, with only thirty-seven of these coded by the mitochondrial genome, indicating heavy mitochondrial dependence on nuclear loci. The mitochondrial genome codes for a complement of 24 genes, including 2 rRNAs and 22 tRNAs that ensure correct translation of the remaining 13 genes which are vital to electron transport (see Figure 1). The mitochondrial genome is dependent on seventy nuclear encoded proteins to accomplish the oxidation and reduction reactions necessary for this vital function, in addition to the thirteen polypeptides supplied by the mitochondrial genome. Both nuclear and mitochondrial proteins form complexes spanning the inner mitochondrial membrane and collectively generate 80-90% of the chemical fuel adenosine triphosphate, or ATP, required for cellular metabolism. In addition to energy production, mitochondria play a central role in other metabolic pathways as well. A critical function of the mitochondria is mediation of cell death, or

1 apoptosis (see Green and Kroemer, 2005). Essentially, there are signal pathways which
2 permeabilize the outer mitochondrial membrane, or in addition, the inner mitochondrial membrane as
3 well. When particular mitochondrial proteins are released into the cytosol, non-reversible cell death
4 is set in motion. This process highlights the multi-functional role that some mitochondrial proteins
5 have. These multi-tasking proteins suggest that there are other mitochondrial proteins as well which
6 may have alternate functions.

7 **[0006]** Mitochondrial Fusion Transcriptome

8 **[0007]** The mitochondrial genome is unusual in that it is a circular, intron-less DNA molecule.
9 The genome is interspersed with repeat motifs which flank specific lengths of sequences.
10 Sequences between these repeats are prone to deletion under circumstances which are not well
11 understood. Given the number of repeats in the mitochondrial genome, there are many possible
12 deletions. The best known example is the 4977 “common deletion.” This deletion has been
13 associated with several purported conditions and diseases and is thought to increase in frequency
14 with aging (Dai et al., 2004; Ro et al., 2003; Barron et al., 2001; Lewis et al., 2000; Muller-Hocker,
15 1998; Porteous et al., 1998) (Figure 4). The current thinking in the field of mitochondrial genomics is
16 that mitochondrial deletions are merely deleterious by-products of damage to the mitochondrial
17 genome by such agents as reactive oxygen species and UVR. (Krishnan et al 2008, Nature
18 Genetics). Further, though it is recognized that high levels of mtDNA deletions can have severe
19 consequences on the cell’s ability to produce energy in the form of ATP as a result of missing gene
20 sequences necessary for cellular respiration, it is not anticipated that these deleted mitochondrial
21 molecules may be a component of downstream pathways, have an intended functional role, and
22 possibly may be more aptly viewed as alternate natural forms of the recognized genes of the
23 mitochondria as has been anticipated by the Applicant.

24 **[0008]** The sequence dynamics of mtDNA are important diagnostic tools. Mutations in mtDNA
25 are often preliminary indicators of developing disease. For example, it has been demonstrated that
26 point mutations in the mitochondrial genome are characteristic of tumour foci in the prostate. This
27 trend also extends to normal appearing tissue both adjacent to and distant from tumour tissue (Parr
28 et al. 2006). This suggests that mitochondrial mutations occur early in the malignant transformation
29 pathway.

30 **[0009]** For example, the frequency of a 3.4kb mitochondrial deletion has excellent utility in
31 discriminating between benign and malignant prostate tissues (Maki et al. 2008).

32 **[0010]** Mitochondrial fusion transcripts have been reported previously in the literature, first in
33 soybeans (Morgens et al. 1984) and then later in two patients with Kearns-Sayre Syndrome, a rare

1 neuromuscular disorder (Nakase et al 1990). Importantly, these transcripts were not found to have
2 (or investigated regarding) association with any human cancers.

3 **SUMMARY OF THE INVENTION**

4 **[0011]** An object of the present invention to provide aberrant mitochondrial DNA, associated
5 fusion transcripts and hybridization probes therefor.

6 **[0012]** In accordance with an aspect of the invention, there is provided an isolated mitochondrial
7 fusion transcript associated with cancer.

8 **[0013]** In accordance with an aspect of the invention, there is provided a mitochondrial fusion
9 protein corresponding to the above fusion transcript, having a sequence as set forth in any one of
10 SEQ ID NOs: 34 to 49 and 52.

11 **[0014]** In accordance with another aspect of the invention, there is provided an isolated mtDNA
12 encoding a fusion transcript of the invention.

13 **[0015]** In accordance with another aspect of the invention, there is provided a hybridization
14 probe having a nucleic acid sequence complementary to at least a portion of a mitochondrial fusion
15 transcript or an mtDNA of the invention.

16 **[0016]** In accordance with another aspect of the invention, there is provided a method of
17 detecting a cancer in a mammal, the method comprising assaying a tissue sample from the mammal
18 for the presence of at least one mitochondrial fusion transcript associated with cancer by hybridizing
19 the sample with at least one hybridization probe having a nucleic acid sequence complementary to
20 at least a portion of a mitochondrial fusion transcript according to the invention.

21 **[0017]** In accordance with another aspect of the invention, there is provided a method of
22 detecting a cancer in a mammal, the method comprising assaying a tissue sample from the mammal
23 for the presence of at least one aberrant mtDNA associated with cancer by hybridizing the sample
24 with at least one hybridization probe having a nucleic acid sequence complementary to at least a
25 portion of an mtDNA according to the invention.

26 **[0018]** In accordance with another aspect of the invention, there is provided a kit for conducting
27 an assay for detecting the presence of a cancer in a mammal, said kit comprising at least one
28 hybridization probe complementary to at least a portion of a fusion transcript or an mtDNA of the
29 invention.

30 **[0019]** In accordance with another aspect of the invention, there is provided a screening tool
31 comprised of a microarray having 10's, 100's, or 1000's of mitochondrial fusion transcripts for
32 identification of those associated with cancer.

1 **[0020]** In accordance with another aspect of the invention, there is provided a screening tool
2 comprised of a microarray having 10's, 100's, or 1000's of mitochondrial DNAs corresponding to
3 mitochondrial fusion transcripts for identification of those associated with cancer.

4 **[0021]** In accordance with another aspect of the invention, there is provided a screening tool
5 comprised of a multiplexed branched DNA assay having 10's, 100's, or 1000's of mitochondrial
6 fusion transcripts for identification of those associated with cancer.

7 **[0022]** In accordance with another aspect of the invention, there is provided a screening tool
8 comprised of a multiplexed branched DNA assay having 10's, 100's, or 1000's of mitochondrial
9 DNAs corresponding to mitochondrial fusion transcripts for identification of those associated with
10 cancer.

11 **BRIEF DESCRIPTION OF THE DRAWINGS**

12 **[0023]** The embodiments of the invention will now be described by way of example only with
13 reference to the appended drawings wherein:

14 **[0024]** Figure 1 is an illustration showing mitochondrial coding genes.

15 **[0025]** Figure 2 shows polyadenalated fusion transcripts in prostate samples invoked by the loss
16 of the 3.4kb deletion.

17 **[0026]** Figure 3 shows polyadenalated fusion transcripts in prostate samples invoked by the loss
18 of the 4977kb common deletion.

19 **[0027]** Figure 4 shows polyadenalated fusion transcripts in breast samples invoked by the loss
20 of the 3.4 kb segment from the mtgenome.

21 **[0028]** Figures 5a and 5b show an example of a mitochondrial DNA region before and after
22 splicing of genes.

23 **[0029]** Figures 6a to 6g illustrate the results for transcripts 2, 3, 8, 9, 10, 11 and 12 of the
24 invention in the identification of colorectal cancer tumours.

25 **[0030]** Figures 7a to 7d illustrate the results for transcripts 6, 8, 10 and 20 of the invention in the
26 identification of lung cancer tumours.

27 **[0031]** Figures 8a to 8g illustrate the results for transcripts 6, 10, 11, 14, 15, 16 and 20 of the
28 invention in the identification of melanomas.

29 **[0032]** Figures 9a to 9h illustrate the results for transcripts 1, 2, 3, 6, 11, 12, 15 and 20 of the
30 invention in the identification of ovarian cancer.

1 **[0033]** Figures 10 to 18 illustrate the results for transcripts 2, 3, 4, 11, 12, 13, 15, 16 and 20 of
2 the invention in the identification of testicular cancer.

3 **DETAILED DESCRIPTION OF THE INVENTION**

4 **[0034]** The present invention provides novel mitochondrial fusion transcripts and the parent
5 mutated mtDNA molecules that are useful for predicting, diagnosing and/or monitoring cancer. The
6 invention further provides hybridization probes for the detection of fusion transcripts and associated
7 mtDNA molecules and the use of such probes.

8 **[0035]** Definitions

9 **[0036]** Unless defined otherwise, all technical and scientific terms used herein have the same
10 meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

11 **[0037]** As used herein, "aberration" or "mutation" encompasses any modification in the wild type
12 mitochondrial DNA sequence that results in a fusion transcript and includes, without limitation,
13 insertions, translocations, deletions, duplications, recombinations, rearrangements or combinations
14 thereof.

15 **[0038]** As defined herein, "biological sample" refers to a tissue or bodily fluid containing cells
16 from which a molecule of interest can be obtained. For example, the biological sample can be
17 derived from tissue such as prostate, breast, colorectal, lung and skin, or from blood, saliva, cerebral
18 spinal fluid, sputa, urine, mucous, synovial fluid, peritoneal fluid, amniotic fluid and the like. The
19 biological sample may be a surgical specimen or a biopsy specimen. The biological sample can be
20 used either directly as obtained from the source or following a pre-treatment to modify the character
21 of the sample. Thus, the biological sample can be pre-treated prior to use by, for example,
22 preparing plasma or serum from blood, disrupting cells, preparing liquids from solid materials,
23 diluting viscous fluids, filtering liquids, distilling liquids, concentrating liquids, inactivating interfering
24 components, adding reagents, and the like.

25 **[0039]** A "continuous" transcript is a fusion transcript that keeps the reading frame from the
26 beginning to the end of both spliced genes. An "end" transcript is a fusion transcript that results in a
27 premature termination codon before the original termination codon of a second spliced gene.

28 **[0040]** As used herein, "mitochondrial DNA" or "mtDNA" is DNA present in mitochondria.

29 **[0041]** As used herein, the expression "mitochondrial fusion transcript" or "fusion transcript"
30 refers to an RNA transcription product produced as a result of the transcription of a mutated
31 mitochondrial DNA sequence wherein such mutations may comprise mitochondrial deletions and
32 other large-scale mitochondrial DNA rearrangements.

1 **[0042]** Computer Analysis and Sequence Targetting

2 **[0043]** As discussed above, mitochondrial fusion transcripts have been reported in soybeans
3 (Morgens et al. 1984) and in humans suffering from a rare neuromuscular disorder (Nakase et al
4 1990). Fusion transcripts associated with human cancer have not, however, been described.

5 **[0044]** Using the knowledge gained from mapping the large-scale deletions of the human
6 mitochondrial genome associated with cancer, the observation of high frequencies of these
7 deletions, and the evidence in another organism and another disease type of transcriptionally active
8 mutated mtDNA molecules, Applicant hypothesized that such deletions may have importance
9 beyond the DNA molecule and the damage and repair processes as it relates to cancer. To test this
10 hypothesis computer analysis of the mitochondrial genome was conducted, specific for repeat
11 elements, which suggested many potential deletion sites. Following this initial step identifying
12 unique repeats in the mitochondrial sequence having non-adjacent or non-tandem locations, a filter
13 was then applied to identify those repeats that upon initiating a deletion event in the DNA molecule
14 would then likely reclose or religate to produce a fused DNA sequence having an open reading
15 frame (ORF). A subset of 18 molecules were then selected for targetting to investigate whether: 1)
16 they existed in the natural biological state of humans and 2) they had relevance to malignancy.
17 Results from these investigations are described hereinafter.

18 **[0045]** Genomic Mutations

19 **[0046]** Mitochondrial DNA (mtDNA) dynamics are an important diagnostic tool. Mutations in
20 mtDNA are often preliminary indicators of developing disease and behave as biomarkers indicative
21 of risk factors associated with disease onset. According to the present invention, large-scale
22 rearrangement mutations in the mitochondrial genome result in the generation of fusion transcripts
23 associated with cancer. Thus, the use of mtDNA encoding such transcripts and probes directed
24 thereto for the detection, diagnosis and monitoring of cancer is provided.

25 **[0047]** One of skill in the art will appreciate that the mtDNA molecules for use in the methods of
26 the present invention may be derived through the isolation of naturally-occurring mutants or may be
27 based on the complementary sequence of any of the fusion transcripts described herein. Exemplary
28 mtDNA sequences and fusion transcripts are disclosed in Applicant's U.S. priority application no.
29 61/040,616, herein incorporated in its entirety by reference.

30 **[0048]** Detection of Mutant Genomic Sequences

31 **[0049]** Mutant mtDNA sequences according to the present invention may comprise any
32 modification that results in the generation of a fusion transcript. Non-limiting examples of such
33 modifications include insertions, translocations, deletions, duplications, recombinations,

1 rearrangements or combinations thereof. While the modification or change can vary greatly in size
2 from only a few bases to several kilobases, preferably the modification results in a substantive
3 deletion or other large-scale genomic aberration.

4 **[0050]** Extraction of DNA to detect the presence of such mutations may take place using art-
5 recognized methods, followed by amplification of all or a region of the mitochondrial genome, and
6 may include sequencing of the mitochondrial genome, as described in Current Protocols in
7 Molecular Biology. Alternatively, crude tissue homogenates may be used as well as techniques not
8 requiring amplification of specific fragments of interest.

9 **[0051]** The step of detecting the mutations can be selected from any technique as is known to
10 those skilled in the art. For example, analyzing mtDNA can comprise selection of targets by
11 branching DNA, sequencing the mtDNA, amplifying mtDNA by PCR, Southern, Northern, Western
12 South-Western blot hybridizations, denaturing HPLC, hybridization to microarrays, biochips or gene
13 chips, molecular marker analysis, biosensors, melting temperature profiling or a combination of any
14 of the above.

15 **[0052]** Any suitable means to sequence mitochondrial DNA may be used. Preferably, mtDNA is
16 amplified by PCR prior to sequencing. The method of PCR is well known in the art and may be
17 performed as described in Mullis and Faloona, 1987, Methods Enzymol., 155: 335. PCR products
18 can be sequenced directly or cloned into a vector which is then placed into a bacterial host.
19 Examples of DNA sequencing methods are found in Brumley, R. L. Jr. and Smith, L.M., 1991, Rapid
20 DNA sequencing by horizontal ultrathin gel electrophoresis, Nucleic Acids Res. 19:4121-4126 and
21 Luckey, J.A., et al, 1993, High speed DNA sequencing by capillary gel electrophoresis, Methods
22 Enzymol. 218: 154-172. The combined use of PCR and sequencing of mtDNA is described in
23 Hopgood, R., et al, 1992, Strategies for automated sequencing of human mtDNA directly from PCR
24 products, Biotechniques 13:82-92 and Tanaka, M. et al, 1996, Automated sequencing of mtDNA,
25 Methods Enzymol. 264: 407-421.

26 **[0053]** Methods of selecting appropriate sequences for preparing various primers are also
27 known in the art. For example, the primer can be prepared using conventional solid-phase synthesis
28 using commercially available equipment, such as that available from Applied Biosystems USA Inc.
29 (Foster City, California), DuPont, (Wilmington, Del.), or Milligen (Bedford, Mass.).

30 **[0054]** According to an aspect of the invention, to determine candidate genomic sequences, a
31 junction point of a sequence deletion is first identified. Sequence deletions are primarily identified by
32 direct and indirect repetitive elements which flank the sequence to be deleted at the 5' and 3' end.

1 The removal of a section of the nucleotides from the genome followed by the ligation of the genome
2 results in the creation of a novel junction point.

3 **[0055]** Upon identification of the junction point, the nucleotides of the genes flanking the junction
4 point are determined in order to identify a spliced gene. Typically the spliced gene comprises the
5 initiation codon from the first gene and the termination codon of the second gene, and may be
6 expressed as a continuous transcript, i.e. one that keeps the reading frame from the beginning to the
7 end of both spliced genes. It is also possible that alternate initiation or termination codons contained
8 within the gene sequences may be used as is evidenced by SEQ ID No:2 and SEQ ID No: 17
9 disclosed herein. Some known mitochondrial deletions discovered to have an open reading frame
10 (ORF) when the rearranged sequences are rejoined at the splice site are provided in Table 1.

11 **[0056]** Exemplary mtDNA molecules for use in the methods of the present invention, which have
12 been verified to exist in the lab, are provided below. These mtDNAs are based on modifications of
13 the known mitochondrial genome (SEQ ID NO: 1) and have been assigned a fusion or "FUS"
14 designation, wherein A:B represents the junction point between the last mitochondrial nucleotide of
15 the first spliced gene and the first mitochondrial nucleotide of the second spliced gene. The
16 identification of the spliced genes is provided in parentheses followed by the corresponding
17 sequence identifier. Where provided below, (AltMet) and (OrigMet) refer to alternate and original
18 translation start sites, respectively.

19 FUS 8469:13447 (AltMet) (ATP synthase F0 subunit 8 to NADH dehydrogenase subunit)
20 (SEQ ID No: 2)

21 FUS 10744:14124 (NADH dehydrogenase subunit 4L (ND4L) to NADH dehydrogenase
22 subunit 5 (ND5)) (SEQ ID No: 3)

23 FUS 7974:15496 (Cytochrome c oxidase subunit II (COII) to Cytochrome b (Cytb)) (SEQ ID
24 No: 4)

25 FUS 7992:15730 (Cytochrome c oxidase subunit II (COII) to Cytochrome b (Cytb)) (SEQ ID
26 No: 5)

27 FUS 8210:15339 (Cytochrome c oxidase subunit II (COII) to Cytochrome b (Cytb)) (SEQ ID
28 No: 6)

29 FUS 8828:14896 (ATP synthase F0 subunit 6 (ATPase6) to Cytochrome b (Cytb)) (SEQ ID
30 No: 7)

31 FUS 10665:14856 (NADH dehydrogenase subunit 4L (ND4L) to Cytochrome b (Cytb)) (SEQ
32 ID No: 8)

1 FUS 6075:13799 (Cytochrome c oxidase subunit I (COI) to NADH de hydrogenase subunit 5
2 (ND5)) (SEQ ID No: 9)

3 FUS 6325:13989 (Cytochrome c oxidase subunit I (COI) to NADH dehydrogenase subunit 5
4 (ND5)) (SEQ ID No: 10)

5 FUS 7438:13476 (Cytochrome c oxidase subunit I (COI) to NADH dehydrogenase subunit 5
6 (ND5)) (SEQ ID No: 11)

7 FUS 7775:13532 (Cytochrome c oxidase subunit II (COII) to NADH dehydrogenase subunit 5
8 (ND5)) (SEQ ID No: 12)

9 FUS 8213:13991 (Cytochrome c oxidase subunit II (COII) to NADH dehydrogenase subunit 5
10 (ND5)) (SEQ ID No: 13)

11 FUS 9191:12909 (ATP synthase F0 subunit 6 (ATPase6) to NADH dehydrogenase subunit 5
12 (ND5)) (SEQ ID No: 14)

13 FUS 9574:12972 (Cytochrome c oxidase subunit III (COIII) to NADH dehydrogenase subunit
14 5 (ND5)) (SEQ ID No: 15)

15 FUS 10367:12829 (NADH dehydrogenase subunit 3 (ND3) to NADH dehydrogenase subunit
16 5 (ND5)) (SEQ ID No: 16)

17 FUS 8469:13447 (OrigMet) (ATP synthase F0 subunit 8 to NADH dehydrogenase subunit)
18 (SEQ ID No: 17)

19 FUS 9144:13816 ((ATP synthase F0 subunit 6 (ATPase6) to NADH dehydrogenase subunit
20 5 (ND5)) (SEQ ID No: 51)

21 **[0057]** The present invention also provides the use of variants or fragments of these sequences
22 for predicting, diagnosing and/or monitoring cancer.

23 **[0058]** "Variant", as used herein, refers to a nucleic acid differing from a mtDNA sequence of the
24 present invention, but retaining essential properties thereof. Generally, variants are overall closely
25 similar, and, in many regions, identical to a select mtDNA sequence. Specifically, the variants of the
26 present invention comprise at least one of the nucleotides of the junction point of the spliced genes,
27 and may further comprise one or more nucleotides adjacent thereto. In one embodiment of the
28 invention, the variant sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to
29 any one of the mtDNA sequences of the invention, or the complementary strand thereto.

30 **[0059]** In the present invention, "fragment" refers to a short nucleic acid sequence which is a
31 portion of that contained in the disclosed genomic sequences, or the complementary strand thereto.

1 This portion includes at least one of the nucleotides comprising the junction point of the spliced
2 genes, and may further comprise one or more nucleotides adjacent thereto. The fragments of the
3 invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more
4 preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt,
5 at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for
6 example, is intended to include 20 or more contiguous bases of any one of the mtDNA sequences
7 listed above. In this context "about" includes the particularly recited value, a value larger or smaller
8 by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These fragments have
9 uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of
10 course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are also contemplated.

11 **[0060]** Thus, in specific embodiments of the invention, the mtDNA sequences are selected from
12 the group consisting of:

- 13 SEQ ID NO: 2 (FUS 8469:13447; AltMet)
- 14 SEQ ID NO: 3 (FUS 10744:14124)
- 15 SEQ ID NO: 4 (FUS 7974:15496)
- 16 SEQ ID NO: 5 (FUS 7992:15730)
- 17 SEQ ID NO: 6 (FUS 8210:15339)
- 18 SEQ ID NO: 7 (FUS 8828:14896)
- 19 SEQ ID NO: 8 (FUS 10665:14856)
- 20 SEQ ID NO: 9 (FUS 6075:13799)
- 21 SEQ ID NO: 10 (FUS 6325:13989)
- 22 SEQ ID NO: 11 (FUS 7438:13476)
- 23 SEQ ID NO: 12 (FUS 7775:13532)
- 24 SEQ ID NO: 13 (FUS 8213:13991)
- 25 SEQ ID NO: 14 (FUS 9191:12909)
- 26 SEQ ID NO: 15 (FUS 9574:12972)
- 27 SEQ ID NO: 16 (FUS 10367:12829)
- 28 SEQ ID NO: 17 (FUS 8469:13447; OrigMet)
- 29 SEQ ID NO: 51 (FUS 9144:13816), and

1 fragments or variants thereof.

2 **[0061]** Probes

3 **[0062]** Another aspect of the invention is to provide a hybridization probe capable of recognizing
4 an aberrant mtDNA sequence of the invention. As used herein, the term "probe" refers to an
5 oligonucleotide which forms a duplex structure with a sequence in the target nucleic acid, due to
6 complementarity of at least one sequence in the probe with a sequence in the target region. The
7 probe may be labeled, according to methods known in the art.

8 **[0063]** Once aberrant mtDNA associated with a particular disease is identified, hybridization of
9 mtDNA to, for example, an array of oligonucleotides can be used to identify particular mutations,
10 however, any known method of hybridization may be used.

11 **[0064]** As with the primers of the present invention, probes may be generated directly against
12 exemplary mtDNA fusion molecules of the invention, or to a fragment or variant thereof. For
13 instance, the sequences set forth in SEQ ID NOs: 2-17 and 51 and those disclosed in Table 1 can
14 be used to design primers or probes that will detect a nucleic acid sequence comprising a fusion
15 sequence of interest. As would be understood by those of skill in the art, primers or probes which
16 hybridize to these nucleic acid molecules may do so under highly stringent hybridization conditions
17 or lower stringency conditions, such conditions known to those skilled in the art and found, for
18 example, in Current Protocols in Molecular Biology (John Wiley & Sons, New York (1989)), 6.3.1-
19 6.3.6.

20 **[0065]** In specific embodiments of the invention, the probes of the invention contain a sequence
21 complementary to at least a portion of the aberrant mtDNA comprising the junction point of the
22 spliced genes. This portion includes at least one of the nucleotides involved in the junction point A:B,
23 and may further comprise one or more nucleotides adjacent thereto. In this regard, the present
24 invention encompasses any suitable targeting mechanism that will select an mtDNA molecule using
25 the nucleotides involved and/or adjacent to the junction point A:B.

26 **[0066]** Various types of probes known in the art are contemplated by the present invention. For
27 example, the probe may be a hybridization probe, the binding of which to a target nucleotide
28 sequence can be detected using a general DNA binding dye such as ethidium bromide, SYBR®
29 Green, SYBR® Gold and the like. Alternatively, the probe can incorporate one or more detectable
30 labels. Detectable labels are molecules or moieties a property or characteristic of which can be
31 detected directly or indirectly and are chosen such that the ability of the probe to hybridize with its
32 target sequence is not affected. Methods of labelling nucleic acid sequences are well-known in the

1 art (see, for example, Ausubel et al., (1997 & updates) Current Protocols in Molecular Biology, Wiley
2 & Sons, New York).

3 **[0067]** Labels suitable for use with the probes of the present invention include those that can be
4 directly detected, such as radioisotopes, fluorophores, chemiluminophores, enzymes, colloidal
5 particles, fluorescent microparticles, and the like. One skilled in the art will understand that directly
6 detectable labels may require additional components, such as substrates, triggering reagents, light,
7 and the like to enable detection of the label. The present invention also contemplates the use of
8 labels that are detected indirectly.

9 **[0068]** The probes of the invention are preferably at least about 15 nt, and more preferably at
10 least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about
11 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A probe of "at least
12 20 nt in length," for example, is intended to include 20 or more contiguous bases that are
13 complementary to an mtDNA sequence of the invention. Of course, larger probes (e.g., 50, 150, 500,
14 600, 2000 nucleotides) may be preferable.

15 **[0069]** The probes of the invention will also hybridize to nucleic acid molecules in biological
16 samples, thereby enabling the methods of the invention. Accordingly, in one aspect of the invention,
17 there is provided a hybridization probe for use in the detection of cancer, wherein the probe is
18 complementary to at least a portion of an aberrant mtDNA molecule. In another aspect the present
19 invention provides probes and a use of (or a method of using) such probes for the detection of
20 colorectal cancer, lung cancer, breast cancer, ovarian cancer, testicular, cancer, prostate cancer
21 and/or melanoma skin cancer.

22 **[0070]** Assays

23 **[0071]** Measuring the level of aberrant mtDNA in a biological sample can determine the
24 presence of one or more cancers in a subject. The present invention, therefore, encompasses
25 methods for predicting, diagnosing or monitoring cancer, comprising obtaining one or more biological
26 samples, extracting mtDNA from the samples, and assaying the samples for aberrant mtDNA by:
27 quantifying the amount of one or more aberrant mtDNA sequences in the sample and comparing the
28 quantity detected with a reference value. As would be understood by those of skill in the art, the
29 reference value is based on whether the method seeks to predict, diagnose or monitor cancer.
30 Accordingly, the reference value may relate to mtDNA data collected from one or more known non-
31 cancerous biological samples, from one or more known cancerous biological samples, and/or from
32 one or more biological samples taken over time.

1 **[0072]** In one aspect, the invention provides a method of detecting cancer in a mammal, the
2 method comprising assaying a tissue sample from the mammal for the presence of an aberrant
3 mitochondrial DNA described above. The present invention also provides for methods comprising
4 assaying a tissue sample from the mammal by hybridizing the sample with at least one hybridization
5 probe. The probe may be generated against a mutant mitochondrial DNA sequence of the invention
6 as described herein.

7 **[0073]** In another aspect, the invention provides a method as above, wherein the assay
8 comprises:

9 a) conducting a hybridization reaction using at least one of the probes to allow the at least
10 one probe to hybridize to a complementary aberrant mitochondrial DNA sequence;

11 b) quantifying the amount of the at least one aberrant mitochondrial DNA sequence in the
12 sample by quantifying the amount of the mitochondrial DNA hybridized to the at least one probe;
13 and,

14 c) comparing the amount of the mitochondrial DNA in the sample to at least one known
15 reference value.

16 **[0074]** Also included in the present invention are methods for predicting, diagnosing or
17 monitoring cancer comprising diagnostic imaging assays as described below. The diagnostic assays
18 of the invention can be readily adapted for high-throughput. High-throughput assays provide the
19 advantage of processing many samples simultaneously and significantly decrease the time required
20 to screen a large number of samples. The present invention, therefore, contemplates the use of the
21 nucleotides of the present invention in high-throughput screening or assays to detect and/or
22 quantitate target nucleotide sequences in a plurality of test samples.

23 **[0075]** Fusion Transcripts

24 **[0076]** The present invention further provides the identification of fusion transcripts and
25 associated hybridization probes useful in methods for predicting, diagnosing and/or monitoring
26 cancer. One of skill in the art will appreciate that such molecules may be derived through the
27 isolation of naturally-occurring transcripts or, alternatively, by the recombinant expression of mtDNAs
28 isolated according to the methods of the invention. As discussed, such mtDNAs typically comprise a
29 spliced gene having the initiation codon from the first gene and the termination codon of the second
30 gene. Accordingly, fusion transcripts derived therefrom comprise a junction point associated with the
31 spliced genes.

32 **[0077]** Detection of Fusion Transcripts

1 **[0078]** Naturally occurring fusion transcripts can be extracted from a biological sample and
2 identified according to any suitable method known in the art, or may be conducted according to the
3 methods described in the examples. In one embodiment of the invention, stable polyadenylated
4 fusion transcripts are identified using Oligo(dT) primers that target transcripts with poly-A tails,
5 followed by RT-PCR using primer pairs designed against the target transcript.

6 **[0079]** The following exemplary fusion transcripts were detected using such methods and found
7 useful in predicting, diagnosing and/or monitoring cancer as indicated in the examples. Likewise,
8 fusion transcripts derived from the ORF sequences identified in Table 1 may be useful in predicting,
9 diagnosing and/or monitoring cancer according to the assays and methods of the present invention.

10 SEQ ID NO: 18 (Transcripts 1;8469:13447; AltMet)

11 SEQ ID NO: 19 (Transcript 2;10744:14124)

12 SEQ ID NO: 20 (Transcript 3;7974:15496)

13 SEQ ID NO: 21 (Transcript 4;7992:15730)

14 SEQ ID NO: 22 (Transcript 5;8210:15339)

15 SEQ ID NO: 23 (Transcript 6;8828:14896)

16 SEQ ID NO: 24 (Transcript 7;10665:14856)

17 SEQ ID NO: 25 (Transcript 8;6075:13799)

18 SEQ ID NO: 26 (Transcript 9;6325:13989)

19 SEQ ID NO: 27 (Transcript 10;7438:13476)

20 SEQ ID NO: 28 (Transcript 11;7775:13532)

21 SEQ ID NO: 29 (Transcript 12;8213:13991)

22 SEQ ID NO: 30 (Transcript 14;9191:12909)

23 SEQ ID NO: 31 (Transcript 15;9574:12972)

24 SEQ ID NO: 32 (Transcript 16;10367:12829)

25 SEQ ID NO: 33 (Transcript 20;8469:13447; OrigMet)

26 SEQ ID NO: 50 (Transcript 13; 9144:13816)

27 **[0080]** Further, fusion transcripts of like character to those described herein are contemplated
28 for use in the field of clinical oncology.

1 **[0081]** Fusion transcripts can also be produced by recombinant techniques known in the art.
2 Typically this involves transformation (including transfection, transduction, or infection) of a suitable
3 host cell with an expression vector comprising an mtDNA sequence of interest.

4 **[0082]** Variants or fragments of the fusion transcripts identified herein are also provided. Such
5 sequences may adhere to the size limitations and percent identities described above with respect to
6 genomic variants and fragments, or as determined suitable by a skilled technician.

7 **[0083]** In addition, putative protein sequences corresponding to transcripts 1-16 and 20 are
8 listed below. These sequences, which encode hypothetical fusion proteins, are provided as a further
9 embodiment of the present invention.

10 SEQ ID NO: 34 (Transcripts 1)

11 SEQ ID NO: 35 (Transcript 2)

12 SEQ ID NO: 36 (Transcript 3)

13 SEQ ID NO: 37 (Transcript 4)

14 SEQ ID NO: 38 (Transcript 5)

15 SEQ ID NO: 39 (Transcript 6)

16 SEQ ID NO: 40 (Transcript 7)

17 SEQ ID NO: 41 (Transcript 8)

18 SEQ ID NO: 42 (Transcript 9)

19 SEQ ID NO: 43 (Transcript 10)

20 SEQ ID NO: 44 (Transcript 11)

21 SEQ ID NO: 45 (Transcript 12)

22 SEQ ID NO: 46 (Transcript 14)

23 SEQ ID NO: 47 (Transcript 15)

24 SEQ ID NO: 48 (Transcript 16)

25 SEQ ID NO: 49 (Transcripts 20)

26 SEQ ID NO: 52 (Transcript 13)

27 **[0084]** Probes

1 **[0085]** Once a fusion transcript has been characterized, primers or probes can be developed to
2 target the transcript in a biological sample. Such primers and probes may be prepared using any
3 known method (as described above) or as set out in the examples provided below. A probe may, for
4 example, be generated for the fusion transcript, and detection technologies, such as QuantiGene
5 2.0TM by Panomics TM, used to detect the presence of the transcript in a sample. Primers and
6 probes may be generated directly against exemplary fusion transcripts of the invention, or to a
7 fragment or variant thereof. For instance, the sequences set forth in SEQ ID NOs: 18-33 and 50 as
8 well as those disclosed in Table 1 can be used to design probes that will detect a nucleic acid
9 sequence comprising a fusion sequence of interest.

10 **[0086]** As would be understood by those skilled in the art, probes designed to hybridize to the
11 fusion transcripts of the invention contain a sequence complementary to at least a portion of the
12 transcript expressing the junction point of the spliced genes. This portion includes at least one of the
13 nucleotides complementary to the expressed junction point, and may further comprise one or more
14 complementary nucleotides adjacent thereto. In this regard, the present invention encompasses any
15 suitable targeting mechanism that will select a fusion transcript that uses the nucleotides involved
16 and adjacent to the junction point of the spliced genes.

17 **[0087]** Various types of probes and methods of labelling known in the art are contemplated for
18 the preparation of transcript probes. Such types and methods have been described above with
19 respect to the detection of genomic sequences. The transcript probes of the invention are preferably
20 at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30
21 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at
22 least about 150 nt in length. A probe of "at least 20 nt in length," for example, is intended to include
23 20 or more contiguous bases that are complementary to an mtDNA sequence of the invention. Of
24 course, larger probes (e.g., 50, 150, 500, 600, 2000 nucleotides) may be preferable.

25 **[0088]** In one aspect, the invention provides a hybridization probe for use in the detection of
26 cancer, wherein the probe is complementary to at least a portion of a mitochondrial fusion transcript
27 provided above.

28 **[0089]** In another aspect, the present invention provides probes and a use of (or a method of
29 using) such probes for the detection of colorectal cancer, lung cancer, breast cancer, ovarian
30 cancer, testicular cancer, prostate cancer or melanoma skin cancer.

31 **[0090]** Assays

32 **[0091]** Measuring the level of mitochondrial fusion transcripts in a biological sample can
33 determine the presence of one or more cancers in a subject. The present invention, therefore,

1 provides methods for predicting, diagnosing or monitoring cancer, comprising obtaining one or more
2 biological samples, extracting mitochondrial RNA from the samples, and assaying the samples for
3 fusion transcripts by: quantifying the amount of one or more fusion transcripts in the sample and
4 comparing the quantity detected with a reference value. As would be understood by those of skill in
5 the art, the reference value is based on whether the method seeks to predict, diagnose or monitor
6 cancer. Accordingly, the reference value may relate to transcript data collected from one or more
7 known non-cancerous biological samples, from one or more known cancerous biological samples,
8 and/or from one or more biological samples taken over time.

9 **[0092]** In one aspect, the invention provides a method of detecting a cancer in a mammal, the
10 method comprising assaying a tissue sample from said mammal for the presence of at least one
11 fusion transcript of the invention by hybridizing said sample with at least one hybridization probe
12 having a nucleic acid sequence complementary to at least a portion of the mitochondrial fusion
13 transcript.

14 **[0093]** In another aspect, the invention provides a method as above, wherein the assay
15 comprises:

16 a) conducting a hybridization reaction using at least one of the above-noted probes to allow
17 the at least one probe to hybridize to a complementary mitochondrial fusion transcript;

18 b) quantifying the amount of the at least one mitochondrial fusion transcript in the sample by
19 quantifying the amount of the transcript hybridized to the at least one probe; and,

20 c) comparing the amount of the mitochondrial fusion transcript in the sample to at least one
21 known reference value.

22 **[0094]** As discussed above, the diagnostic assays of the invention may also comprise
23 diagnostic methods and screening tools as described herein and can be readily adapted for high-
24 throughput. The present invention, therefore, contemplates the use of the fusion transcripts and
25 associated probes of the present invention in high-throughput screening or assays to detect and/or
26 quantitate target nucleotide sequences in a plurality of test samples.

27 **[0095]** Diagnostic Methods and Screening Tools

28 **[0096]** Methods and screening tools for diagnosing specific diseases or identifying specific
29 mitochondrial mutations are also herein contemplated. Any known method of hybridization may be
30 used to carry out such methods including, without limitation, probe/primer based technologies such
31 as branched DNA and qPCR, both single-plex and multi-plex. Array technology, which has
32 oligonucleotide probes matching the wild type or mutated region, and a control probe, may also be

1 used. Commercially available arrays such as microarrays or gene chips are suitable. These arrays
2 contain thousands of matched and control pairs of probes on a slide or microchip, and are capable
3 of sequencing the entire genome very quickly. Review articles describing the use of microarrays in
4 genome and DNA sequence analysis are available on-line.

5 **[0097]** Screening tools designed to identify targets which are relevant to a given biological
6 condition may include specific arrangements of nucleic acids associated with a particular disease or
7 disorder. Thus, in accordance with one embodiment of the invention, there is provided a screening
8 tool comprised of a microarray having 10's, 100's, or 1000's of mitochondrial fusion transcripts for
9 identification of those associated with one or more cancers. In accordance with another
10 embodiment, there is provided a screening tool comprised of a microarray having 10's, 100's, or
11 1000's of mitochondrial DNAs corresponding to mitochondrial fusion transcripts for identification of
12 those associated with one or more cancers. In a further embodiment, there is provided a screening
13 tool comprised of a multiplexed branched DNA assay having 10's, 100's, or 1000's of mitochondrial
14 fusion transcripts for identification of those associated with one or more cancers. In yet another
15 embodiment of the invention, there is provided a screening tool comprised of a multiplexed branched
16 DNA assay having 10's, 100's, or 1000's of mitochondrial DNAs corresponding to mitochondrial
17 fusion transcripts for identification of those associated with one or more cancers.

18 **[0098]** Approaches useful in the field of clinical oncology are also herein contemplated and may
19 include such diagnostic imaging techniques as Positron Emission Tomography (PET), contrast
20 Magnetic Resonance Imaging (MRI) or the like. These diagnostic methods are well known to those
21 of skill in the art and are useful in the diagnosis and prognosis of cancer.

22 **[0099]** Diagnostic Monitoring

23 **[00100]** The methods of the present invention may further comprise the step of recommending a
24 monitoring regime or course of therapy based on the outcome of one or more assays. This allows
25 clinicians to practice personalized medicine; e.g. cancer therapy, by monitoring the progression of
26 the patient's cancer (such as by recognizing when an initial or subsequent mutation occurs) or
27 treatment (such as by recognizing when a mutation is stabilized).

28 **[00101]** With knowledge of the boundaries of the sequence variation in hand, the information can
29 be used to diagnose a pre-cancerous condition or existing cancer condition. Further, by quantitating
30 the amount of aberrant mtDNA in successive samples over time, the progression of a cancer
31 condition can be monitored. For example, data provided by assaying the patient's tissues at one
32 point in time to detect a first set of mutations from wild-type could be compared against data
33 provided from a subsequent assay, to determine if changes in the aberration have occurred.

1 **[00102]** Where a mutation is found in an individual who has not yet developed symptoms of
2 cancer, the mutation may be indicative of a genetic susceptibility to develop a cancer condition. A
3 determination of susceptibility to disease or diagnosis of its presence can further be evaluated on a
4 qualitative basis based on information concerning the prevalence, if any, of the cancer condition in
5 the patient's family history and the presence of other risk factors, such as exposure to environmental
6 factors and whether the patient's cells also carry a mutation of another sort.

7 **[00103]** Biological Sample

8 **[00104]** The present invention provides for diagnostic tests which involve obtaining or collecting
9 one or more biological samples. In the context of the present invention, "biological sample" refers to
10 a tissue or bodily fluid containing cells from which mtDNA and mtRNA can be obtained. For
11 example, the biological sample can be derived from tissue including, but not limited to, skin, lung,
12 breast, prostate, nervous, muscle, heart, stomach, colon, rectal tissue and the like; or from blood,
13 saliva, cerebral spinal fluid, sputa, urine, mucous, synovial fluid, peritoneal fluid, amniotic fluid and
14 the like. The biological sample may be obtained from a cancerous or non-cancerous tissue and may
15 be, but is not limited to, a surgical specimen or a biopsy specimen.

16 **[00105]** The biological sample can be used either directly as obtained from the source or
17 following a pre-treatment to modify the character of the sample. Thus, the biological sample can be
18 pre-treated prior to use by, for example, preparing plasma or serum from blood, disrupting cells,
19 preparing liquids from solid materials, diluting viscous fluids, filtering liquids, distilling liquids,
20 concentrating liquids, inactivating interfering components, adding reagents, and the like.

21 **[00106]** One skilled in the art will understand that more than one sample type may be assayed at
22 a single time (i.e. for the detection of more than one cancer). Furthermore, where a course of
23 collections are required, for example, for the monitoring of cancer over time, a given sample may be
24 diagnosed alone or together with other samples taken throughout a test period. In this regard,
25 biological samples may be taken once only, or at regular intervals such as biweekly, monthly, semi-
26 annually or annually.

27 **[00107]** Kits

28 **[00108]** The present invention provides diagnostic/screening kits for detecting cancer in a clinical
29 environment. Such kits may include one or more sampling means, in combination with one or more
30 probes according to the present invention.

31 **[00109]** The kits can optionally include reagents required to conduct a diagnostic assay, such as
32 buffers, salts, detection reagents, and the like. Other components, such as buffers and solutions for
33 the isolation and/or treatment of a biological sample, may also be included in the kit. One or more of

1 the components of the kit may be lyophilised and the kit may further comprise reagents suitable for
2 the reconstitution of the lyophilised components.

3 **[00110]** Where appropriate, the kit may also contain reaction vessels, mixing vessels and other
4 components that facilitate the preparation of the test sample. The kit may also optionally include
5 instructions for use, which may be provided in paper form or in computer-readable form, such as a
6 disc, CD, DVD or the like.

7 **[00111]** In one embodiment of the invention there is provided a kit for diagnosing cancer
8 comprising sampling means and a hybridization probe of the invention.

9 **[00112]** Various aspects of the invention will be described by illustration using the following
10 examples. The examples provided herein serve only to illustrate certain specific embodiments of the
11 invention and are not intended to limit the scope of the invention in any way.

12 **EXAMPLES**

13 **[00113]** Example 1: Detection of Mitochondrial Fusion Transcripts

14 **[00114]** The mitochondrial 4977 “common deletion” and a 3.4kb deletion previously identified by
15 the present Applicant in PCT application no. PCT/CA2007/001711 (the entire contents of which are
16 incorporated by reference) result in unique open reading frames having active transcripts as
17 identified by oligo-dT selection in prostate tissue (Figures 2 and 3). Examination of breast tissue
18 samples also reveals the presence of a stable polyadenylated fusion transcript resulting from the
19 3.4kb deletion (Figure 4).

20 **[00115]** Reverse transcriptase-PCR protocol for deletion transcript detection

21 **[00116]** RNA isolation cDNA synthesis

22 **[00117]** Total RNA was isolated from snap frozen prostate and breast tissue samples (both
23 malignant and normal samples adjacent to tumours) using the Aurum™ Total RNA Fatty and
24 Fibrous Tissue kit (Bio-Rad, Hercules, CA) following the manufacturer’s instructions. Since in this
25 experiment, genomic DNA contamination was to be avoided, a DNase I treatment step was included,
26 using methods as commonly known in the art. RNA quantity and quality were determined with an
27 ND-1000 spectrophotometer (NanoDrop® technologies). From a starting material of about 100g,
28 total RNA concentrations varied from 100 – 1000ng/ul with a 260/280 ratio between 1.89 – 2.10.
29 RNA concentrations were adjusted to 100ng/ul and 2ul of each template were used for first strand
30 DNA synthesis with SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen) following
31 the manufacturer’s instructions. In order to identify stable polyadenylated fusion transcripts,
32 Oligo(dT) primers that target transcripts with poly-A tails were used.

1 **[00118]** PCR

2 **[00119]** Real time PCR was performed using 5ul of each cDNA template with the iQ™ SYBR®
3 Green Supermix (Bio-Rad, Hercules, CA) on DNA Engine Opticon® 2 Continuous Fluorescence
4 Detection System (Bio-Rad, Hercules, CA). The primer pairs targeting the 4977bp deletion are;
5 8416F 5'- CCTTACACTATTCCTCATCAC- 3', 13637R 5'- TGACCTGTTAGGGTGAGAAG - 3', and
6 those for the 3.4 kb deletion are; ND4LF 5'- TCGCTCACACCTCATATCCTC -3', ND5R 5'-
7 TGTGATTAGGAGTAGGGTTAGG -3'. The reaction cocktail included: 2X SYBR® Green Supermix
8 (100mM KCL, 40mM Tris-HCl, pH 8.4, 0.4mM of each dNTP [dATP, dCTP, dGTP, and dTTP],
9 iTaq™ DNA polymerase, 50 units/ml, 6mM MgCl₂, SYBR® Green 1, 20nM fluorescein, and
10 stabilizers), 250nM each of primers, and ddH₂O. PCR cycling parameters were as follows; (1) 95°C
11 for 2 min, (2) 95°C for 30 sec, (3) 55°C (for the 4977bp deletion) and 63°C (for the 3.4 kb deletion)
12 for 30 sec , (4) 72°C for 45 sec, (5) plate read, followed by 39 cycles of steps 3 to 5, and final
13 incubation at 4°C. Apart from cycling threshold and melting curve analysis, samples were run on
14 agarose gels for specific visualization of amplification products (see Figures 2 to 4).

15 **[00120]** Figure 2 is an agarose gel showing polyadenalated fusion transcripts in prostate samples
16 invoked by the loss of 3.4kb from the mitochondrial genome. Legend for Figure 2: B-blank, Lanes 1-
17 6 transcripts detected in cDNA; lanes 7-12 no reverse transcriptase (RT) controls for samples in
18 lanes 1-6.

19 **[00121]** Figure 3 shows polyadenalated fusion transcripts in prostate samples invoked by the loss
20 of the 4977kb common deletion. Legend for Figure 3: B-blank, Lanes 1-6 transcripts detected in
21 cDNA; lanes 7-12 no RT controls for samples in lanes 1-6.

22 **[00122]** Figure 4 shows polyadenalated fusion transcripts in breast samples invoked by the loss
23 of 3.4kb from the mtgenome. Legend for Figure 4: Lanes 2-8 transcripts from breast cDNAs; lane 9
24 negative (water) control; lanes 10 and 11, negative, no RT, controls for samples in lanes 2 and 3.

25 **[00123]** These results demonstrate the existence of stable mitochondrial fusion transcripts.

26 **[00124]** Example 2: Identification and Targetting of Fusion Products

27 **[00125]** Various hybridization probes were designed to detect, and further demonstrate the
28 presence of novel transcripts resulting from mutated mitochondrial genomes, such as the 3.4kb
29 deletion. For this purpose, a single-plex branched DNA platform for quantitative gene expression
30 analysis (QuantiGene 2.0™, Panomics™) was utilized. The specific deletions and sequences listed
31 in this example are based on their relative positions with the entire mtDNA genome, which is recited
32 in SEQ ID NO: 1. The nucleic acid sequences of the four transcript to which the probes were

designed in this example are identified herein as follows: Transcript 1 (SEQ ID NO: 18), Transcript 2 (SEQ ID NO: 19), Transcript 3 (SEQ ID NO: 20) and Transcript 4 (SEQ ID NO: 21).

[00126] An example of a continuous transcript from the 3.4kb mitochondrial genome deletion occurs with the genes ND4L (NADH dehydrogenase subunit 4L) and ND5 (NADH dehydrogenase subunit 5). A probe having a complementary sequence to SEQ ID NO: 19, was used to detect transcript 2. The repetitive elements occur at positions 10745-10754 in ND4L and 14124-14133 in ND5.

[00127] The 3.4kb deletion results in the removal of the 3' end of ND4L, the full ND4 gene, tRNA histidine, tRNA serine2, tRNA leucine2, and the majority of the 5' end of ND5 (see Figure 5a), resulting in a gene splice of ND4L and ND5 with a junction point of 10744(ND4L):14124(ND5) (Figure 5b). SEQ ID NO: 3 is the complementary DNA sequence to the RNA transcript (SEQ ID NO: 19) detected in the manner described above.

[00128] Similarly, transcript 1 is a fusion transcript between ATPase 8 and ND5 associated with positions 8469:13447 (SEQ ID NO: 18). Transcripts 3 and 4 (SEQ ID NO: 20 and SEQ ID NO: 21, respectively) are fusion transcripts between COII and Cytb associated with nucleotide positions 7974:15496 and 7992:15730 respectively. Table 3 provides a summary of the relationships between the various sequences used in this example. Table 3 includes the detected fusion transcript and the DNA sequence complementary to the fusion transcript detected.

[00129] Example 3: Application to Prostate Cancer

[00130] Using the four fusion transcripts, i.e. transcripts 1 to 4, discussed above, two prostate tissue samples from one patient were analyzed to assess the quantitative difference of the novel predicted fusion transcripts. The results of the experiment are provided in Table 2 below, wherein "Homog 1" refers to the homogenate of frozen prostate tumour tissue from a patient and "Homog 2" refers to the homogenate of frozen normal prostate tissue adjacent to the tumour of the patient. These samples were processed according to the manufacturer's protocol (QuantiGene® Sample Processing Kit for Fresh or Frozen Animal Tissues; and QuantiGene® 2.0 Reagent System User Manual) starting with 25.8 mg of Homog 1 and 28.9 mg of Homog 2 (the assay setup is shown in Tables 5a and 5b).

[00131] Clearly demonstrated is an increased presence of mitochondrial fusion transcripts in prostate cancer tissue compared to normal adjacent prostate tissue. The fusion transcript is present in the normal tissue, although at much lower levels. The relative luminescence units (RLU) generated by hybridization of a probe to a target transcript are directly proportional to the abundance of each transcript. Table 2 also indicates the coefficients of variation, CV, expressed as a

percentage, of the readings taken for the samples. The CV comprises the Standard deviation divided by the average of the values. The significance of such stably transcribed mitochondrial gene products in cancer tissue has implications in disease evolution and progression.

[00132] Example 4: Application to Breast Cancer

[00133] Using the same protocol from Example 3 but focusing only on Transcript 2, the novel fusion transcript associated with the 3.4kb mtgenome deletion, analyses were conducted on two samples of breast tumour tissue and two samples of tumour-free tissues adjacent to those tumours, as well as three samples of prostate tumour tissue, one sample comprising adjacent tumour-free tissue. Results for this example are provided in Table 4. The prostate tumour tissue sample having a corresponding normal tissue section demonstrated a similar pattern to the prostate sample analyzed in Example 3 in that the tumour tissue had approximately 2 times the amount of the fusion transcript than did the normal adjacent tissue. The breast tumour samples demonstrated a marked increase in the fusion transcript levels when compared to the adjacent non-tumour tissues. A 1:100 dilution of the homogenate was used for this analysis as it performed most reproducibly in the experiment cited in Example 3.

[00134] Thus, the above discussed results illustrate the application of the transcripts of the invention in the detection of tumours of both prostate and breast tissue.

[00135] Example 5: Application to Colorectal Cancer

[00136] This study sought to determine the effectiveness of several transcripts of the invention in detecting colorectal cancer. A total of 19 samples were prepared comprising nine control (benign) tissue samples (samples 1 to 9) and ten tumour (malignant) tissue samples (samples 10 to 19). The samples were homogenized according to the manufacturer's recommendations (Quantigene® Sample Processing Kit for Fresh or Frozen Animal Tissues; and Quantigene 2.0 Reagent System User Manual). Seven target transcripts and one housekeeper transcript were prepared in the manner as outlined above in previous examples. The characteristics of the transcripts are summarized as follows:

[00137] Table 7: Characteristics of Breast Cancer Transcripts

Transcript ID	Junction Site	Gene Junction
2	10744:14124	ND4L:ND5
3	7974:15496	COII:Cytb
10	7438:13476	COI:ND5
11	7775:13532	COII:ND5
12	8213:13991	COII:ND5
Peptidylpropyl isomerase B (PPIB) ("housekeeper")	N/A	N/A

1

2 **[00138]** It is noted that transcripts 2 and 3 are the same as those discussed above with respect to
3 Examples 3 and 4.

4 **[00139]** Homogenates were prepared using approximately 25mg of tissue from OCT blocks and
5 diluted 1:1 for transcripts 2 and 4, and 1:8 for transcripts 10 and 11. The quantity of the transcripts
6 was measured in Relative Luminescence Units RLU on a Glomax™ Multi Detection System
7 (Promega). All samples were assayed in triplicate for each transcript. Background measurements
8 (no template) were done in triplicate as well. The analysis accounted for background by subtracting
9 the lower limit from the RLU values for the samples. Input RNA was accounted for by using the
10 formula $\log_2 a \text{ RLU} - \log_2 h \text{ RLU}$ where a is the target fusion transcript and h is the housekeeper
11 transcript.

12 **[00140]** The analysis of the data comprised the following steps:

13 a) Establish CV's (coefficients of variation) for triplicate assays; acceptable if $\leq 15\%$.

14 b) Establish average RLU value for triplicate assays of target fusion transcript(a) and
15 housekeeper transcript (h).

16 c) Establish lower limit from triplicate value of background RLU (l).

17 d) Subtract lower limit (l) from (a).

18 e) Calculate $\log_2 a \text{ RLU} - \log_2 h \text{ RLU}$.

19 **[00141]** Summary of Results:

20 **[00142]** The results of the above analysis are illustrated in Figures 6a to 6g, which comprise plots
21 of the $\log_2 a \text{ RLU} - \log_2 h \text{ RLU}$ against sample number. Also illustrated are the respective ROC
22 (Receiver Operating Characteristic) curves determined from the results for each transcript.

23 **[00143]** Transcript 2: There exists a statistically significant difference between the means
24 ($p < 0.10$) of the normal and malignant groups ($p > 0.09$), using a cutoff value of 3.6129 as
25 demonstrated by the ROC curve results in a sensitivity of 60% and specificity of 89% and the area
26 under the curve is 0.73 indicating fair test accuracy. The threshold value chosen may be adjusted to
27 increase either the specificity or sensitivity of the test for a particular application.

28 **[00144]** Transcript 3: There exists a statistically significant difference between the means
29 ($p < 0.05$) of the normal and malignant groups ($p = 0.03$), using a cutoff value of 4.0813 as
30 demonstrated by the ROC curve results in a sensitivity of 60% and specificity of 78% and the area

1 under the curve is 0.79 indicating fair to good test accuracy. The threshold value chosen may be
2 adjusted to increase either the specificity or sensitivity of the test for a particular application.

3 **[00145]** Transcript 8: There exists a statistically significant difference between the means
4 ($p < 0.1$) of the normal and malignant groups ($p = 0.06$). Using a cutoff value of -6.0975 as
5 demonstrated by the ROC curve results in a sensitivity of 60% and specificity of 89% and the area
6 under the curve is 0.76 indicating fair test accuracy. The threshold value chosen may be adjusted
7 to increase either the specificity or sensitivity of the test for a particular application.

8 **[00146]** Transcript 9: There exists a statistically significant difference between the means
9 ($p < 0.1$) of the normal and malignant groups ($p = 0.06$). Using a cutoff value of -7.5555 as
10 demonstrated by the ROC curve results in a sensitivity of 60% and specificity of 89% and the area
11 under the curve is 0.76 indicating fair to good test accuracy. The threshold value chosen may be
12 adjusted to increase either the specificity or sensitivity of the test for a particular application.

13 **[00147]** Transcript 10: There is a statistically significant difference between the means
14 ($p \leq 0.01$) of the normal and malignant groups ($p = 0.01$). Using a cutoff value of -3.8272 as
15 demonstrated by the ROC curve results in a sensitivity of 90% and specificity of 67% and the area
16 under the curve is 0.84, indicating good test accuracy. The threshold value chosen may be adjusted
17 to increase either the specificity or sensitivity of the test for a particular application.

18 **[00148]** Transcript 11: There exists a statistically significant difference between the means
19 ($p < 0.1$) of the normal and malignant groups ($p = 0.06$), using a cutoff value of 3.1753 as
20 demonstrated by the ROC curve results in a sensitivity of 70% and specificity of 78% and the area
21 under the curve is 0.76 indicating fair to good test accuracy. The threshold value chosen may be
22 adjusted to increase either the specificity or sensitivity of the test for a particular application.

23 **[00149]** Transcript 12: There exists a statistically significant difference between the means
24 ($p < 0.1$) of the normal and malignant groups ($p = 0.06$), using a cut-off value of 3.2626 as
25 demonstrated by the ROC curve results in a sensitivity of 70% and specificity of 78% and the area
26 under the curve is 0.76 indicating fair to good test accuracy. The threshold value chosen may be
27 adjusted to increase either the specificity or sensitivity of the test for a particular application.

28 **[00150]** Conclusions:

29 **[00151]** The above results illustrate the utility of transcripts 2, 3, 8, 9, 10, 11, and 12 in the
30 detection of colorectal cancer and in distinguishing malignant from normal colorectal tissue. As
31 indicated above, transcripts 2 and 3 were also found to have utility in the detection of prostate
32 cancer. Transcript 2 was also found to have utility in the detection of breast cancer. Transcript 11
33 was also found to have utility in the detection of melanoma skin cancer. Transcript 10 was also

found to have utility in the detection of lung cancer and melanoma. Transcript 8 was also found to have utility in the detection of lung cancer. Any of the 7 transcripts listed may be used individually or in combination as a tool for the detection of characterization of colorectal cancer in a clinical setting.

[00152] Example 6: Application to Lung Cancer

[00153] This study sought to determine the effectiveness of several transcripts of the invention in the detection of lung cancer. As in Example 5, nine control (benign) tissue samples (samples 1 to 9) and ten tumour (malignant) tissue samples (samples 10 to 19) were homogenized according to the manufacturer's recommendations (Quantigene® Sample Processing Kit for Fresh or Frozen Animal Tissues; and Quantigene 2.0 Reagent System User Manual). Homogenates were diluted 1:8 and the quantity of 4 target transcripts and 1 housekeeper transcript was measured in Relative Luminescence Units RLU on a Glomax™ Multi Detection System (Promega). All samples were assayed in triplicate for each transcript. Background measurements (no template) were done in triplicate as well.

[00154] The following transcripts were prepared for this example:

[00155] Table 8: Characteristics of Lung Cancer Transcripts

Transcript ID	Junction Site	Gene Junction
6	8828:14896	ATPase6:Cytb
8	6075:13799	COI:ND5
10	7438:13476	COI:ND5
20	8469:13447	ATPase8:ND5
Peptidylpropyl isomerase B (PPIB) ("housekeeper")	N/A	N/A

[00156] The tissue samples used in this example had the following characteristics:

[00157] Table 9: Characteristics of Lung Cancer Samples

Sample	Malignant	Comments (source of tissue)
1	NO	interstitial lung disease
2	NO	emphysema
3	NO	aneurysm
4	NO	bronchopneumonia, COPD
5	NO	malignant neoplasm in liver, origin unknown, calcified granulomas in lung
6	NO	12 hours post mortem, mild emphysema
7	NO	12 hours post mortem, large B cell lymphoma, pulmonary edema, pneumonia
8	NO	pneumonia, edema, alveolar damage
9	NO	congestion and edema
10	YES	adenocarcinoma, non-small cell
11	YES	small cell

12	YES	squamous cell carcinoma, NSC, emphysema
13	YES	adenocarcinoma, lung cancer, nsc, metastatic
14	YES	squamous cell carcinoma, non-small cell
15	YES	mixed squamous and adenocarcinoma
16	YES	non-small cell carcinoma, squamous
17	YES	small cell carcinoma
18	YES	adenocarcinoma, lung cancer, nsc
19	YES	adenocarcinoma, lung cancer, nsc, metastatic

1

2 **[00158]** The analysis of data was performed according to the method described in Example 5.

3 The results are illustrated in figures 7a, 7b, 7c and 7d.

4 **[00159]** Summary of Results:

5 **[00160]** Transcript 6: There exists a statistically significant difference between the means
6 ($p < 0.1$) of the normal (benign) and malignant groups ($p = 0.06$), using a cutoff value of -6.5691 as
7 demonstrated by the ROC curve results in a sensitivity of 80% and specificity of 71% and the area
8 under the curve is 0.77, indicating fair test accuracy. The threshold value chosen may be adjusted
9 to increase either the specificity or sensitivity of the test for a particular application.

10 **[00161]** Transcript 8: The difference between the means of the normal and malignant
11 groups is statistically significant, $p < 0.05$ ($p = 0.02$). Using a cutoff value of -9.6166 as demonstrated
12 by the ROC curve results in a sensitivity of 90% and specificity of 86% and the area under the curve
13 is 0.86 indicating good test accuracy. The threshold value chosen may be adjusted to increase
14 either the specificity or sensitivity of the test for a particular application.

15 **[00162]** Transcript 10: The difference between the means of the normal and malignant
16 groups is statistically significant, $p \leq 0.01$ ($p = 0.01$). Using a cutoff value of -10.6717 as demonstrated
17 by the ROC curve results in a sensitivity of 90% and specificity of 86% and the area under the curve
18 is 0.89 indicating good test accuracy. The threshold value chosen may be adjusted to increase
19 either the specificity or sensitivity of the test for a particular application.

20 **[00163]** Transcript 20: The difference between the means of the normal and malignant
21 groups is statistically significant, $p \leq 0.1$ ($p = 0.1$). Using a cutoff value of 2.5071 as demonstrated by
22 the ROC curve results in a sensitivity of 70% and specificity of 71% and the area under the curve is
23 0.74 indicating fair test accuracy. The threshold value chosen may be adjusted to increase either
24 the specificity or sensitivity of the test for a particular application.

25 **[00164]** Conclusions:

26 **[00165]** The results from example 6 illustrate the utility of transcripts 6, 8, 10, and 20 of the
27 invention in the detection of lung cancer tumours and the distinction between malignant and normal

1 lung tissues. Any of these three transcripts may be used for the detection or characterization of lung
2 cancer in a clinical setting.

3 **[00166]** Example 7: Application to Melanoma

4 **[00167]** This study sought to determine the effectiveness of several transcripts of the invention in
5 the detection of melanomas. In this study a total of 14 samples were used, comprising five control
6 (benign) tissue samples and nine malignant tissue samples. All samples were formalin fixed,
7 paraffin embedded (FFPE). The FFPE tissue samples were sectioned into tubes and homogenized
8 according to the manufacturer's recommendations (Quantigene® 2.0 Sample Processing Kit for
9 FFPE Samples; and Quantigene 2.0 Reagent System User Manual) such that each sample
10 approximated 20 microns prior to homogenization. Homogenates were diluted 1:4 and the quantity
11 of 7 target transcripts and 1 housekeeper transcript was measured in Relative Luminescence Units
12 RLU on a Glomax™ Multi Detection System (Promega). All samples were assayed in triplicate for
13 each transcript. Background measurements (no template) were done in triplicate as well.

14 **[00168]** The 14 tissue samples used in this example had the following characteristics:

15 **[00169]** Table 10: Characteristics of Melanoma Cancer Samples

Sample	Malignant	Comments (source of tissue)
1	NO	breast reduction tissue (skin)
2	NO	breast reduction tissue (skin)
3	NO	breast reduction tissue (skin)
4	NO	breast reduction tissue (skin)
5	NO	breast reduction tissue (skin)
6	YES	lentigo maligna, (melanoma in situ) invasive melanoma not present
7	YES	invasive malignant melanoma
8	YES	nodular melanoma, pT3b, associated features of lentigo maligna
9	YES	residual superficial spreading invasive malignant melanoma, Clark's level II
10	YES	superficial spreading malignant melanoma, Clark's Level II
11	YES	nodular malignant melanoma, Clark's level IV
12	YES	superficial spreading malignant melanoma in situ, no evidence of invasion
13	YES	superficial spreading malignant melanoma, Clark's level II, focally present vertical phase
14	YES	superficial spreading malignant melanoma in situ, Clark's level I

16

17 **[00170]** The following transcripts were prepared for this example:

18 **[00171]** Table 11: Characteristics of Melanoma Cancer Transcripts

Transcript ID	Junction Site	GeneJunction
6	8828:4896	ATPase6:Cytb
10	7438:13476	COI:ND5
11	7775:13532	COII:ND5

14	9191:12909	ATPase6:ND5
15	9574:12972	COIII:ND5
16	10367:12829	ND3:ND5
20	8469:13447	ATPase8:ND5
Peptidylpropyl isomerase B (PPIB) ("housekeeper")	N/A	N/A

1

2 **[00172]** As indicated, transcripts 10 and 11 were also used in Example 5. The analysis of data
3 was performed according to the method described in Example 5. The results are illustrated in
4 figures 8a -8g.

5 **[00173]** Summary of Results:

6 **[00174]** Transcript 6: There exists a statistically significant difference between the means
7 ($p \leq 0.01$) of the normal and malignant groups ($p = 0.01$). Further, using a cutoff value of -5.9531 as
8 demonstrated by the ROC curve results in a sensitivity of 89% and specificity of 80% and the area
9 under the curve is 0.96, indicating very good test accuracy. The threshold value chosen may be
10 adjusted to increase either the specificity or sensitivity of the test for a particular application.

11 **[00175]** Transcript 10: There exists a statistically significant difference between the means
12 ($p \leq 0.05$) of the normal and malignant groups ($p = 0.05$), using a cutoff value of -4.7572as
13 demonstrated by the ROC curve results in a sensitivity of 89% and specificity of 40% and the area
14 under the curve is 0.82, indicating good test accuracy. The threshold value chosen may be adjusted
15 to increase either the specificity or sensitivity of the test for a particular application.

16 **[00176]** Transcript 11: There exists a statistically significant difference between the means
17 ($p < 0.05$) of the normal and malignant groups ($p = 0.02$). Further, using a cutoff value of 1.6762 as
18 demonstrated by the ROC curve results in a sensitivity of 78% and specificity of 100% and the area
19 under the curve is 0.89, indicating good test accuracy. The threshold value chosen may be adjusted
20 to increase either the specificity or sensitivity of the test for a particular application.

21 **[00177]** Transcript 14: There exists a statistically significant difference between the means
22 ($p \leq 0.05$) of the normal and malignant groups ($p = 0.05$). Further, using a cutoff value of -4.9118 as
23 demonstrated by the ROC curve results in a sensitivity of 89% and specificity of 60% and the area
24 under the curve is 0.82, indicating good test accuracy. The threshold value chosen may be adjusted
25 to increase either the specificity or sensitivity of the test for a particular application.

26 **[00178]** Transcript 15: There exists a statistically significant difference between the means
27 ($p < 0.1$) of the normal and malignant groups ($p = 0.07$), using a cutoff value of -7.3107as
28 demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 67% and the area

under the curve is 0.80, indicating good test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00179] Transcript 16: There exists a statistically significant difference between the means ($p < 0.05$) of the normal and malignant groups ($p = 0.03$). Further, using a cutoff value of -10.5963 as demonstrated by the ROC curve results in a sensitivity of 89% and specificity of 80% and the area under the curve is 0.878, indicating good test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00180] Transcript 20: There exists a statistically significant difference between the means ($p < 0.05$) of the normal and malignant groups ($p = 0.04$). Further, using a cutoff value of -8.3543 as demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 80% and the area under the curve is 0.89, indicating good test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00181] Conclusions:

[00182] The results from example 7 illustrate the utility of transcripts 6, 10, 11, 14, 15, 16 and 20 of the invention in the detection of malignant melanomas. As indicated above, transcripts 10 and 11 were also found have utility in detecting colorectal cancer while transcript 6 has utility in the detection of lung cancer. A transcript summary by disease is provided at Table 6.

[00183] Example 8: Application to Ovarian Cancer

[00184] This study sought to determine the effectiveness of several transcripts of the invention in detecting ovarian cancer. A total of 20 samples were prepared comprising ten control (benign) tissue samples (samples 1 to 10) and ten tumour (malignant) tissue samples (samples 11 to 20). The samples were homogenized according to the manufacturer's recommendations (Quantigene® Sample Processing Kit for Fresh or Frozen Animal Tissues; and Quantigene 2.0 Reagent System User Manual). Eight target transcripts and one housekeeper transcript were prepared in the manner as outlined above in previous examples.

[00185] The 20 tissue samples used in this example had the following characteristics:

[00186] Table 12: Characteristics of Ovarian Cancer Samples

Sample	Diagnosis	Comments
1	Normal	follicular cyst
2	Normal	fibroma
3	Normal	No pathological change in ovaries
4	Normal	follicular cysts
5	Normal	cellular fibroma
6	Normal	benign follicular and simple cysts

7	Normal	leiomyomata, corpora albicantia
8	Normal	copora albicantia and an epithelial inclusions cysts
9	Normal	corpora albicantia
10	Normal	corpora albicantia, surface inclusion cysts, follicular cysts
11	Malignant	high grade poorly differentiated papillary serous carcinoma involving omentum
12	Malignant	endometrioid adenocarcinoma, well to moderately differentiated with focal serous differentiation
13	Malignant	papillary serous carcinoma
14	Malignant	mixed epithelial carcinoma predominantly papillary serous carcinoma
15	Malignant	High grade: serous carcinoma, papillary and solid growth patterns
16	Malignant	High Grade (3/3) Papillary serous carcinoma
17	Malignant	papillary serous carcinoma, high nuclear grade
18	Malignant	Papillary serous cystadenocarcinomas Grade:III
19	Malignant	poorly differentiated papillary serous carcinoma
20	Malignant	Well-differentiated adn carcinoma, Endometrioid type, Grade 1

1

2 **[00187]** The characteristics of the transcripts are summarized as follows:

3 **[00188]** Table 13: Characteristics of Ovarian Cancer Transcripts

Transcript ID	Junction Site	Gene Junction
1	8469:13447	ATPase8:ND5
2	10744:14124	ND4L:ND5
3	7974:15496	COII:Cytb
6	8828:14896	ATPase6:Cytb
11	7775:13532	COII:ND5
12	8213:13991	COII:ND5
15	9574:12972	COIII:ND5
20	8469:13447	ATPase8:ND5
Ribosomal Protein Large PO (LRP) Housekeeper	N/A	N/A

4

1 **[00189]** It is noted that transcripts 1, 2, 3, 6, 11, 12, 15 and 20 are the same as those discussed
2 above with respect to Examples 3-7.

3 **[00190]** Homogenates were prepared using approximately 25mg of frozen tissue and diluted 1:4.
4 The quantity of the transcripts was measured in Relative Luminiscence Units RLU on a Glomax™
5 Multi Detection System (Promega). All samples were assayed in triplicate for each transcript.
6 Background measurements (no template) were done in triplicate as well. The analysis accounted for
7 background by subtracting the lower limit from the RLU values for the samples. Input RNA was
8 accounted for by using the formula $\log_2 a \text{ RLU} - \log_2 h \text{ RLU}$ where a is the target fusion transcript
9 and h is the housekeeper transcript.

10 **[00191]** The analysis of the data comprised the following steps:

- 11 a) Establish CV's (coefficients of variation) for triplicate assays; acceptable if $\leq 15\%$.
- 12 b) Establish average RLU value for triplicate assays of target fusion transcript(a) and
13 housekeeper transcript (h).
- 14 c) Establish lower limit from triplicate value of background RLU (l).
- 15 d) Subtract lower limit (l) from (a).
- 16 e) Calculate $\log_2 a \text{ RLU} - \log_2 h \text{ RLU}$.

17 **[00192]** Summary of Results:

18 **[00193]** The results of the above analysis are illustrated in Figures 9a to 9h, which comprise plots
19 of the $\log_2 a \text{ RLU} - \log_2 h \text{ RLU}$ against sample number. Also illustrated are the respective ROC
20 (Receiver Operating Characteristic) curves determined from the results for each transcript.

21 **[00194]** Transcript 1: There exists a statistically significant difference between the means
22 ($p < 0.05$) of the normal and malignant groups ($p = 0.002$). Using a cutoff value of -11.1503 as
23 demonstrated by the ROC curve results in a sensitivity of 90% and specificity of 80% and the area
24 under the curve is 0.91 indicating very good test accuracy. The threshold value chosen may be
25 adjusted to increase either the specificity or sensitivity of the test for a particular application.

26 **[00195]** Transcript 2: There exists a statistically significant difference between the means
27 ($p < 0.01$) of the normal and malignant groups ($p = 0.001$). Using a cutoff value of 0.6962 as
28 demonstrated by the ROC curve results in a sensitivity of 90% and specificity of 100% and the area
29 under the curve is 0.96 indicating very good test accuracy. The threshold value chosen may be
30 adjusted to increase either the specificity or sensitivity of the test for a particular application.

31 **[00196]** Transcript 3: There exists a statistically significant difference between the means
32 ($p < 0.01$) of the normal and malignant groups ($p = 0.000$). Using a cutoff value of 0.6754 as

demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 100% and the area under the curve is 1.00 indicating excellent test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00197] Transcript 6: There exists a statistically significant difference between the means ($p < 0.01$) of the normal and malignant groups ($p = 0.007$). Using a cutoff value of -9.6479 as demonstrated by the ROC curve results in a sensitivity of 90% and specificity of 70% and the area under the curve is 0.86 indicating good test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00198] Transcript 11: There is a statistically significant difference between the means ($p < 0.01$) of the normal and malignant groups ($p = 0.000$). Using a cutoff value of -1.3794 demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 90% and the area under the curve is 0.99, indicating excellent test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00199] Transcript 12: There exists a statistically significant difference between the means ($p < 0.01$) of the normal and malignant groups ($p = 0.001$). Using a cutoff value of -1.2379 as demonstrated by the ROC curve results in a sensitivity of 90% and specificity of 100% and the area under the curve is 0.96 indicating excellent test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00200] Transcript 15: There exists a statistically significant difference between the means ($p < 0.05$) of the normal and malignant groups ($p = 0.023$). Using a cut-off value of -8.6926 as demonstrated by the ROC curve results in a sensitivity of 70% and specificity of 80% and the area under the curve is 0.80 indicating good test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00201] Transcript 20: There exists a statistically significant difference between the means ($p < 0.01$) of the normal and malignant groups ($p = 0.000$). Using a cut-off value of 0.6521 as demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 100% and the area under the curve is 0.76 indicating fair to good test accuracy. The threshold value chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular application.

[00202] Conclusions:

[00203] The above results illustrate the utility of transcripts 1, 2, 3, 6, 11, 12, 15, and 20 in the detection of ovarian cancer and in distinguishing malignant from normal ovarian tissue. Transcripts 1, 2 and 3 were also found to have utility in the detection of prostate cancer. Transcript 6 was also found to have utility in the detection of melanoma and lung cancer. Transcript 11 was also found to

have utility in the detection of melanoma skin cancer, colorectal cancer and testicular cancer. Transcript 12 was also found to have utility in the detection of colorectal cancer and testicular cancer. Transcript 15 was also found to have utility in the detection of melanoma and testicular cancer. Transcript 20 was also found to have utility in the detection of colorectal cancer, melanoma, and testicular cancer. Any of the 8 transcripts listed may be used individually or in combination as a tool for the detection or characterization of ovarian cancer in a clinical setting.

[00204] Example 9: Application to Testicular Cancer

[00205] This study sought to determine the effectiveness of several transcripts of the invention in detecting testicular cancer. A total of 17 samples were prepared comprising eight control (benign) tissue samples (samples 1 to 8) and 9 tumour (malignant) tissue samples (samples 9 to 17), 5 of the malignant samples were non-seminomas (samples 9-13) and 4 were seminomas (samples 14-17). The samples were homogenized according to the manufacturer's recommendations (Quantigene® Sample Processing Kit for Fresh or Frozen Animal Tissues; and Quantigene 2.0 Reagent System User Manual). 10 target transcripts and one housekeeper transcript were prepared in the manner as outlined above in previous examples.

[00206] The 17 tissue samples used in this example had the following characteristics:

[00207] Table 14: Characteristics of Testicular Cancer Samples

Sample	General Diagnosis	Stratified Malignant Diagnosis
1	Benign	Benign
2	Benign	Benign
3	Benign	Benign
4	Benign	Benign
5	Benign	Benign
6	Benign	Benign
7	Benign	Benign
8	Benign	Benign
9	Malignant	Non-Seminoma
10	Malignant	Non-Seminoma
11	Malignant	Non-Seminoma
12	Malignant	Non-Seminoma
13	Malignant	Non-Seminoma
14	Malignant	Seminoma
15	Malignant	Seminoma

16	Malignant	Seminoma
17	Malignant	Seminoma

1 **[00208]** The characteristics of the transcripts are summarized as follows:

2 **[00209]** Table 15: Characteristics of Testicular Cancer Transcripts

Transcript ID	Junction Site	Gene Junction
2	10744:14124	ND4L:ND5
3	7974:15496	COII:Cytb
4	7992:15730	COII:Cytb
11	7775:13532	COII:ND5
12	8213:13991	COII:ND5
13	9144:13816	ATPase6:ND5
15	9574:12972	COIII:ND5
16	10367:12829	ND3:ND5
20	8469:13447	ATPase8:ND5
Peptidylpropyl isomerase B (PPIB)	N/A	N/A

3

4 **[00210]** It is noted that transcripts 2, 3, 4, 7, 11, 12, 15, 16 and 20 are the same as those
5 discussed above with respect to Examples 3-8.

6 **[00211]** Homogenates were prepared using approximately 25mg of frozen tissue and diluted 1:4.
7 The quantity of the transcripts was measured in Relative Luminescence Units RLU on a Glomax™
8 Multi Detection System (Promega). All samples were assayed in triplicate for each transcript.
9 Background measurements (no template) were done in triplicate as well. The analysis accounted for
10 background by subtracting the lower limit from the RLU values for the samples. Input RNA was
11 accounted for by using the formula $\log_2 a \text{ RLU} - \log_2 h \text{ RLU}$ where a is the target fusion transcript
12 and h is the housekeeper transcript.

13 **[00212]** The analysis of the data comprised the following steps:

- 14 a) Establish CV's (coefficients of variation) for triplicate assays; acceptable if $\leq 15\%$.
15 b) Establish average RLU value for triplicate assays of target fusion transcript(a) and
16 housekeeper transcript (h).
17 c) Establish lower limit from triplicate value of background RLU (l).
18 d) Subtract lower limit (l) from (a).
19 e) Calculate $\log_2 a \text{ RLU} - \log_2 h \text{ RLU}$.

1 **[00213]** Summary of Results:

2 **[00214]** The results of the above analysis are illustrated in Figures 10 to 18, which comprise plots
3 of the $\log_2$ a RLU – $\log_2$ h RLU against sample number. Also illustrated are the respective ROC
4 (Receiver Operating Characteristic) curves determined from the results for each transcript.

5 **[00215]** While some transcripts distinguish between benign and malignant testicular tissue,
6 others demonstrate distinction between the tumour subtypes of seminoma and non-seminoma
7 and/or benign testicular tissue. It is therefore anticipated that combining transcripts from each class
8 will facilitate not only detection of testicular cancer but also classification into subtype of seminoma
9 or non-seminomas.

10 **[00216]** Transcript 2: There exists a statistically significant difference between the means
11 ($p < 0.05$) of the normal group and the malignant seminomas ($p = 0.02$). Using a cutoff value of 1.5621
12 as demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 100% and the
13 area under the curve is 1.00 indicating excellent test accuracy. There also exists a statistically
14 significant difference between the means ($p < 0.05$) of the malignant seminomas and the malignant
15 non-seminomas ($p = 0.024$). Using a cutoff value of 2.1006 as demonstrated by the ROC curve
16 results in a sensitivity of 100% and specificity of 80% and the area under the curve is 0.90 indicating
17 excellent test accuracy. The threshold value chosen may be adjusted to increase either the
18 specificity or sensitivity of the test for a particular application.

19 **[00217]** Transcript 3: There exists a statistically significant difference between the means
20 ($p < 0.05$) of the normal group and the malignant seminomas ($p = 0.018$). Using a cutoff value of 0.969
21 as demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 87.5% and the
22 area under the curve is 0.969 indicating excellent accuracy. There also exists a statistically
23 significant difference between the means ($p < 0.05$) of the malignant seminomas and the malignant
24 non-seminomas ($p = 0.017$). Using a cutoff value of 1.8181 as demonstrated by the ROC curve
25 results in a sensitivity of 100% and specificity of 80% and the area under the curve is 0.9 indicating
26 excellent test accuracy. The threshold value chosen may be adjusted to increase either the
27 specificity or sensitivity of the test for a particular application.

28 **[00218]** Transcript 4: There exists a statistically significant difference between the means
29 ($p < 0.05$) of the normal and malignant groups ($p = 0.034$). Using a cutoff value of -9.7628 as
30 demonstrated by the ROC curve results in a sensitivity of 67% and specificity of 100% and the area
31 under the curve is 0.833 indicating good test accuracy. The threshold value chosen may be
32 adjusted to increase either the specificity or sensitivity of the test for a particular application.

1 **[00219]** Transcript 11: There exists a statistically significant difference between the means
2 ($p < 0.05$) of the normal group and the malignant seminomas ($p = 0.016$). Using a cutoff value of 0.732
3 as demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 100% and the
4 area under the curve is 1.00 indicating excellent test accuracy. There also exists a statistically
5 significant difference between the means ($p < 0.05$) of the malignant seminomas and the malignant
6 non-seminomas ($p = 0.016$). Using a cutoff value of 0.9884 as demonstrated by the ROC curve
7 results in a sensitivity of 100% and specificity of 80% and the area under the curve is 0.90 indicating
8 excellent test accuracy. The threshold value chosen may be adjusted to increase either the
9 specificity or sensitivity of the test for a particular application.

10 **[00220]** Transcript 12: There exists a statistically significant difference between the means
11 ($p < 0.1$) of the normal group and the malignant seminomas ($p = 0.056$). Using a cutoff value of 1.5361
12 as demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 87.5% and the
13 area under the curve is 0.969 indicating excellent test accuracy. There also exists a statistically
14 significant difference between the means ($p < 0.05$) of the malignant seminomas and the malignant
15 non-seminomas ($p = 0.044$). Using a cutoff value of 1.6039 as demonstrated by the ROC curve
16 results in a sensitivity of 100% and specificity of 80% and the area under the curve is 0.9 indicating
17 excellent test accuracy. The threshold value chosen may be adjusted to increase either the
18 specificity or sensitivity of the test for a particular application.

19 **[00221]** Transcript 13: There exists a statistically significant difference between the means
20 ($p < 0.05$) of the normal group and the malignant group ($p = 0.019$). Using a cutoff value of -9.8751 as
21 demonstrated by the ROC curve results in a sensitivity of 87.5% and specificity of 78% and the area
22 under the curve is 0.875 indicating very good test accuracy. There also exists a statistically
23 significant difference between the means ($p < 0.01$) of the malignant non-seminomas and the benign
24 group ($p = 0.000$). Using a cutoff value of -13.9519 as demonstrated by the ROC curve results in a
25 sensitivity of 100% and specificity of 87.5% and the area under the curve is 0.975 indicating
26 excellent test accuracy. There also exists a statistically significant difference between the means
27 ($p < 0.01$) of the malignant seminomas and the malignant non-seminomas ($p = 0.001$). Using a cutoff
28 value of -15.8501 as demonstrated by the ROC curve results in a sensitivity of 100% and specificity
29 of 100% and the area under the curve is 1.00 indicating excellent test accuracy. The threshold value
30 chosen may be adjusted to increase either the specificity or sensitivity of the test for a particular
31 application.

32 **[00222]** Transcript 15: There exists a statistically significant difference between the means
33 ($p < 0.1$) of the normal and malignant groups ($p = 0.065$). Using a cut-off value of -5.4916 as
34 demonstrated by the ROC curve results in a sensitivity of 75% and specificity of 89% and the area

1 under the curve is 0.833 indicating good test accuracy. The threshold value chosen may be
2 adjusted to increase either the specificity or sensitivity of the test for a particular application.

3 **[00223]** Transcript 16: There exists a statistically significant difference between the means
4 ($p < 0.05$) of the normal and malignant groups including both seminomas and non-
5 seminomas ($p = 0.037$). Using a cut-off value of -6.448 as demonstrated by the ROC curve results in
6 a sensitivity of 89% and specificity of 75% and the area under the curve is 0.806 indicating good test
7 accuracy. There also exists a statistically significant difference between the means ($p < 0.05$) of the
8 normal and malignant seminomas ($p = 0.037$). Using a cut-off value of -7.4575 as demonstrated by
9 the ROC curve results in a sensitivity of 100% and specificity of 87.5% and the area under the curve
10 is 0.938 indicating excellent test accuracy. The threshold value chosen may be adjusted to increase
11 either the specificity or sensitivity of the test for a particular application.

12 **[00224]** Transcript 20: There exists a statistically significant difference between the means
13 ($p < 0.01$) of the normal group and the malignant seminomas ($p = 0.006$). Using a cutoff value of
14 1.8364 as demonstrated by the ROC curve results in a sensitivity of 100% and specificity of 100%
15 and the area under the curve is 1.00 indicating excellent test accuracy. There also exists a
16 statistically significant difference between the means ($p < 0.01$) of the malignant seminomas and the
17 malignant non-seminomas ($p = 0.004$). Using a cutoff value of 1.6065 as demonstrated by the ROC
18 curve results in a sensitivity of 100% and specificity of 100% and the area under the curve is 1.00
19 indicating excellent test accuracy. The threshold value chosen may be adjusted to increase either
20 the specificity or sensitivity of the test for a particular application.

21 **[00225]** Conclusions:

22 **[00226]** The above results illustrate the utility of transcripts 2, 3, 4, 11, 12, 13, 15, 16, and 20 in
23 the detection of testicular cancer, and testicular cancer subtypes, and in distinguishing malignant
24 from normal testicular tissue. Transcript 2 was also found to have utility in the detection of prostate,
25 breast, colorectal and ovarian cancer. Transcript 3 was also found to have utility in the detection of
26 prostate, breast, melanoma, colorectal, and ovarian cancers. Transcript 4 was also found to have
27 utility in the detection of prostate and colorectal cancers. Transcript 11 was also found to have utility
28 in the detection of colorectal, melanoma, and ovarian cancers. Transcript 12 was also found to have
29 utility in the detection of colorectal and ovarian cancers. Transcript 15 was also found to have utility
30 in the detection of melanoma and ovarian cancers. Transcript 16 was also found to have utility in
31 the detection of melanoma skin cancer. Transcript 20 was also found to have utility in the detection
32 of colorectal cancer, melanoma, and ovarian cancer. Any of the 9 transcripts listed may be used
33 individually or in combination as a tool for the detection or characterization of testicular cancer in a
34 clinical setting.

[00227] In one aspect, the invention provides a kit for conducting an assay for determining the presence of cancer in a tissue sample. The kit includes the required reagents for conducting the assay as described above. In particular, the kit includes one or more containers containing one or more hybridization probes corresponding to transcripts 1 to 17, and 20 described above. As will be understood, the reagents for conducting the assay may include any necessary buffers, salts, detection reagents etc. Further, the kit may include any necessary sample collection devices, containers etc. for obtaining the needed tissue samples, reagents or materials to prepare the tissue samples for example by homogenization or nucleic acid extraction, and for conducting the subject assay or assays. The kit may also include control tissues or samples to establish or validate acceptable values for diseased or non-diseased tissues.

[00228] Although the invention has been described with reference to certain specific embodiments, various modifications thereof will be apparent to those skilled in the art without departing from the scope of the invention as outlined in the claims appended hereto.

[00229] Bibliography

[00230] The following references, amongst others, were cited in the foregoing description.

<u>Author</u>	<u>Journal</u>	<u>Title</u>	<u>Volume</u>	<u>Date</u>
Anderson et al	Nature	Sequence and Organization of the Human Mitochondrial Genome	290(5806):457-65	1981
Andrews et al	Nat Genet	Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA.	23(2):147	1999
Modica-Napolitano et al	Expert Rev Mol Med	Mitochondria as targets for detection and treatment of cancer	4:1-19	2002
Sherratt et al	Clin Sci (Lond)	Mitochondrial DNA defects: a widening clinical spectrum of disorders.	92(3):225-35	1997
Croteau et al	Mutat Res	Mitochondrial DNA repair pathways.	434(3):137-48	1999
Green and Kroemer	J Clin Invest	Pharmacological manipulation of cell death: clinical applications in sight?	115(10): 2610-2617	2005
Dai et al	Acta Otolaryngol	Correlation of cochlear blood supply with mitochondrial DNA common deletion in presbycusis.	24(2):130-6	2004
Ro et al	Muscle Nerve	Deleted 4977-bp mitochondrial DNA mutation is associated with sporadic amyotrophic lateral sclerosis: a hospital-based case-control study.	28(6):737-43	2003
Barron et al	Invest Ophthalmol Vis Sci	Mitochondrial abnormalities in ageing macular photoreceptors.	42(12):3016-22	2001
Lewis et al	J Pathol	Detection of damage to the mitochondrial genome in the oncocyctic cells of Warthin's tumour.	191(3):274-81	2000

Muller-Hocker et al	Mod Pathol	The common 4977 base pair deletion of mitochondrial DNA preferentially accumulates in the cardiac conduction system of patients with Kearns-Sayre syndrome.	11(3):295-301.	1998
Porteous et al	Eur J Biochem	Bioenergetic consequences of accumulating the common 4977-bp mitochondrial DNA deletion.	257(1):192-201	1998
Parr et al	J Mol Diagn	Somatic mitochondrial DNA mutations in prostate cancer and normal appearing adjacent glands in comparison to age-matched prostate samples without malignant histology.	8(3):312-9.	2006
Maki et al	Am J Clin Pathol	Mitochondrial genome deletion aids in the identification of false- and true-negative prostate needle core biopsy specimens.	129(1):57-66	2008
Nakase et al	Am J Hum Genet	Transcription and translation of deleted mitochondrial genomes in Kearns-Sayre syndrome: implications for pathogenesis.	46(3):418-27.	1990
Libura et al	Blood	Therapy-related acute myeloid leukemia-like MLL rearrangements are induced by etoposide in primary human CD34+ cells and remain stable after clonal expansion.	105(5):2124-31	2005
Meyer et al	Proc Natl Acad Sci U S A	Diagnostic tool for the identification of MLL rearrangements including unknown partner genes.	102(2):449-54	2005
Eguchi et al	Genes Chromosomes Cancer	MLL chimeric protein activation renders cells vulnerable to chromosomal damage: an explanation for the very short latency of infant leukemia.	45(8):754-60	2006
Hayashi et al	Proc Natl Acad Sci U S A	Introduction of disease-related mitochondrial DNA deletions into HeLa cells lacking mitochondrial DNA results in mitochondrial dysfunction	88: 10614-10618	1991

1

2

Table 1: Known mitochondrial deletions having an ORF

Deletion Junction (nt:nt)	Deletion Size (bp)	Repeat Location (nt:nt)	Number of Repeats	References
COX I - ND5				
6075:13799	-7723	6076-6084/13799-13807	D, 9/9	•Mita, S., Rizzuto, R., Moraes, C.T., Shanske, S., Arnaudo, E., Fabrizi, G.M., Koga, Y., DiMauro, S., Schon, E.A. (1990) "Recombination via flanking direct repeats is a major cause of large-scale deletions of human mitochondrial DNA" Nucleic Acids Research 18(3):561-567
6238:14103	-7864	6235-6238/14099-14102	D, 4/4	•Blok, R.B., Thorburn, D.R., Thompson, G.N., Dahl, H.H. (1995) "A topoisomerase II cleavage site is associated with a novel mitochondrial DNA deletion" Human Genetics 95 (1): 75-81
6325:13989	-7663	6326-6341/13889-14004	D, 16/17	•Larsson, N.G., Holme, E., Kristiansson, B., Oldfors, A., Tulinius, M. (1990) "Progressive increase of the mutated mitochondrial DNA fraction in Kearns-Sayre syndrome " Pediatric Research 28 (2): 131-136
6330:13994	-7663	6331-6341/13994-14004	D, 11/11	•Larsson, N.G., Holme, E. (1992) "Multiple short direct repeats associated with single mtDNA deletions " Biochimica et Biophysica Acta 1139 (4): 311-314
COX II - ND5				
7829:14135	-6305	7824-7829/14129-14134	D, 6/6	•Mita, S., Rizzuto, R., Moraes, C.T., Shanske, S., Arnaudo, E., Fabrizi, G.M., Koga, Y., DiMauro, S., Schon, E.A. (1990) "Recombination via flanking direct repeats is a major cause of large-scale deletions of human mitochondrial DNA" Nucleic Acids Research 18(3):561-567
8213:13991	-5777	8214-8220/13991-13997	D, 7/7	•Bet, L., Moggio, M., Comi, G.P., Mariani, C., Prele, A., Checcarelli, N., Bordon, A., Bresolin, N., Scarpini, E., Scarlato, G. (1994) "Multiple sclerosis and mitochondrial myopathy: an unusual combination of diseases" Journal of Neurology 241 (8): 511-516
ATPase - ND5				
8631:13513	-4881	8625-8631/13508-13512	D, 7/7	•Hinokio, Y., Suzuki, S., Komatsu, K., Ohtomo, M., Onoda, M., Matsumoto, M., Hirai, S., Sato, Y., Akai, H., Abe, K., Toyota, T. (1995) "A new mitochondrial DNA deletion associated with diabetic amyotrophy, diabetic myopathy and diabetic fatty liver" Muscle and Nerve 3 (9): S142-149
9144:13816	-4671	9137-9144/13808-13815	D, 8/8	•Zhang, C., Baumer, A., Mackay, I.R., Linnane, A.W., Nagley, P. (1995) "Unusual pattern of mitochondrial DNA deletions in skeletal muscle of an adult human with chronic fatigue syndrome" Human Molecular Genetics 4 (4): 751-754
9191:12909	-3717	9189-9191/12906-12908	D, 3/3	•Ota, Y., Tanaka, M., Sato, W., Ohno, K., Yamamoto, T., Maehara, M., Negoro, T., Watanabe, K., Awaya, S., Ozawa, T. (1991) "Detection of platelet mitochondrial DNA deletions in Kearns-Sayre syndrome" Investigative Ophthalmology and Visual Science 32 (10): 2667-2675
COX III - ND5				
				•Tanaka, M., Sato, W., Ohno, K., Yamamoto, T., Ozawa, T. (1989) "Direct sequencing of mitochondrial DNA in myopathic patients" Biochemical and Biophysical Research Communications 164 (1): 156-163

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10190:13753	-3562	10191-10198M 3753-13760	D, 8/8	•Rotig, A., Bourgeron, T., Chretien, D., Rustin, P., Munnich, A. (1995) "Spectrum of mitochondrial DNA rearrangements in the Pearson marrow-pancreas syndrome" Human Molecular Genetics 4 (8): 1327-1330 •Rotig, A., Cormier, V., Koll, F., Mize, C. E., Saudubray, J.-M., Veerman, A., Pearson, H. A., Munnich, A. (1991) "Site-specific deletions of the mitochondrial genome in Pearson marrow-pancreas syndrome" Genomics 10 (2): 502-504 •Kapsa, R., Thompson, G.N., Thorburn, D.R., Dahl, H.H., Marzuki, S., Byrne, E., Blok, R.B. (1994) "A novel mtDNA deletion in an infant with Pearson syndrome" Journal of Inherited Metabolic Disease 17 (5): 521-526
10367:12829	-2461	10365-10367M 2826-12828	D, 3/3	•Cormier-Daire, V., Bonnefont, J.P., Rustin, P., Maurage, C., Ogier, H., Schmitz, J., Ricour, C., Saudubray, J.M., Munnich, A., Rotig, A. (1994) "Mitochondrial DNA rearrangements with onset as chronic diarrhea with villous atrophy" Journal of Pediatrics 124 (1): 63-70
ND4L - ND5	-3379	10745-10754M 4124-14133	D, 9/10	•Rotig, A., Cormier, V., Koll, F., Mize, C. E., Saudubray, J.-M., Veerman, A., Pearson, H. A., Munnich, A. (1991) "Site-specific deletions of the mitochondrial genome in Pearson marrow-pancreas syndrome" Genomics 10 (2): 502-504 •Rotig, A., Cormier, V., Blanche, S., Bonnefont, J.P., Ledest, F., Romero, N., Schmitz, J., Rustin, P., Fischer, A., Saudubray, J.M. (1990) "Pearson's marrow-pancreas syndrome: A multi-system mitochondrial disorder in infancy" Journal of Clinical Investigation 86 Q: 1601-1608 •Cormier, V., Rotig, A., Quartino, A.R., Forn, G.L., Cerone, R., Maier, M., Saudubray, J.M., Munnich, A. (1990) "Widespread multitissue deletions of the mitochondrial genome in Pearson marrow-pancreas syndrome" Journal of Pediatrics 117 (4): 599-602 •Awata, T., Matsumoto, T., Iwamoto, Y., Matsuda, A., Kuzuya, T., Saito, T. (1993) "Japanese case of diabetes mellitus and deafness with mutations in mitochondrial tRNA^{Leu}(UUR) gene [letter]" Lancet 341 (8855): 1291-1292
10744:14124				
ND4 - ND5				
11232:13980	-2747	11234-11242M 3981-13989	D, 9/9	

Table 2: Prostate Cancer Detection with Novel Mitochondrial Fusion Transcripts

Transcript	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2
	Transcript 1	Transcript 1	Transcript 1	Transcript 2	Transcript 2	Transcript 2	Transcript 3	Transcript 3	Transcript 3	Transcript 4	Transcript 4	Transcript 4
	1	2	3	4	5	6	7	8	9	10	11	12
No dilution	A	2957	353	233	144838	75374	17192	348424	333189	213844	509	565
Replicate A	B	3174	475	298	202793	100062	31750	320877	278137	210265	401	676
1:10 dilution	C	1041	262	114	106195	98403	36191	238467	248677	123497	181	486
Replicate C	D	1040	272	176	120308	116930	50323	239231	262520	129778	153	467
1:100 dilution	E	318	170	110	25155	64823	27725	100345	164606	85287	72	265
Replicate E	F	287	150	109	23500	50524	24629	100856	178527	84731	83	251
1:1000 dilution	G	100	76	123	3002	12960	252	29203	102309	167	31	143
Replicate G	H	94	83	97	1263	5796	285	29092	97257	96	45	110
%CV A		5.0	20.9	17.3	23.6	19.9	42.1	5.8	12.7	1.2	16.9	12.7
%CV C		0.1	2.5	30.1	8.8	12.2	23.1	0.2	3.8	3.5	12.0	2.8
%CV E		7.1	9.0	0.6	4.8	17.5	8.4	0.4	5.7	0.5	9.8	3.8
%CV G		4.7	6.0	20.8	57.7	54.0	8.8	0.3	3.6	25.0	27.0	18.2

* unit results in table are RLU (relative luminescence units); Data read on Glorunner™.

%CV = Coefficient of variation (as %).

Legend: Homog = homogenate.

Homog 1: Prostate tumour tissue sample from patient;

Homog 2: Histologically normal tissue adjacent to tumour from patient.

RNA: Control: Total RNA from prostate tissue (Ambion p/n 7988).

Shading: Background measurement.

Table 3: Deletion/Transcript/DNA Complement

Deletion	RNA transcript	DNA sequence with deletion complementary to RNA transcript	Transcript No.
ATP synthase F0 subunit 8 to NADH dehydrogenase subunit mitochondrial positions 8366-14148 (with reference to SEQ ID NO: 1).	SEQ ID NO: 18	SEQ ID NO: 2	1
NADH dehydrogenase subunit 4L (ND4L) to NADH dehydrogenase subunit 5 (ND5); Mitochondrial positions 10470-14148 (with reference to SEQ ID NO: 1)	SEQ ID NO: 19	SEQ ID NO: 3	2
Cytochrome c oxidase subunit II (COII) to Cytochrome b (Cytb); Mitochondrial positions 7586-15887 (with reference to SEQ ID NO: 1)	SEQ ID NO: 20	SEQ ID NO: 4	3
Cytochrome c oxidase subunit II (COII) to Cytochrome b (Cytb); Mitochondrial positions 7586-15887 (with reference to SEQ ID NO:1)	SEQ ID NO: 21	SEQ ID NO: 5	4

Table 4: Breast and Prostate Cancer Detection

	Breast Tumour 1	Normal adjacent Breast Tumour 1	Breast Tumour 2	Normal Adjacent to Breast Tumour 2	Prostate Tumour 3	Prostate Tumour 4	Prostate Tumour 5	Normal Adjacent to Prostate Tumour 5
	1	2	3	4	5	6	7	8
1:100 dilution	E 68920	2971	49108	1245	46723	56679	99836	35504
1:100 dilution replicate	F 92409	3017	60637	1512	53940	56155	100582	44221
	G 420	3	31	6	26	25	44	23
	H 518	3	4	5	5	3	4	2
	%CV 20.6	1.1	14.9	13.7	10.1	0.7	0.5	15.5

- unit results in table are RLU (relative luminescence units)
- background G1, H1
- empty well G2-G8, H2- H8

Table 5a: Assay Conditions

Template for the assay											
RNA	Homogen 1	Homogen 2	RNA	Homogen 1	Homogen 2	RNA	Homogen 1	Homogen 2	RNA	Homogen 1	Homogen 2
Transcript 1	Transcript 1	Transcript 1	Transcript 2	Transcript 2	Transcript 2	Transcript 3	Transcript 3	Transcript 3	Transcript 4	Transcript 4	Transcript 4
1	2	3	4	5	6	7	8	9	10	11	12
A RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2
B Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1
C RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2
D Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2	Dil 2
E RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2	RNA	Homog 1	Homog 2
F Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3	Dil 3
G RNA	Homog 1	Transcript 1	RNA	Homog 1	Transcript 1	RNA	Homog 1	Transcript 1	RNA	Homog 1	Transcript 1
H Dil 4	Dil 4	Background	Dil 4	Dil 4	Background	Dil 4	Dil 4	Background	Dil 4	Dil 4	Background

Homogenate1- Used 26 mg of tissue to homogenize in 700ul H soln with Proteinase K (PK). Used Qiagen TissueRuptor. Used 40ul homogenate supernatant, 20, 10 and 5 ul for dilution

Homogenate1= Tumour tissue from the tumorous Prostate

Homogenate2- Used 29 mg of tissue to homogenize in 700ul H soln with PK. Used Qiagen TissueRuptor. Used 40ul homogenate supernatant, 20, 10 and 5 ul for dilution

Homogenate2= Normal tissue from the tumorous Prostate

RNA dilution was made as below. RNA was from Prostate Normal from Ambion.
Assay was done in duplicates.

Table 5b: RNA dilution

RNA Dilution	ng/ul
Dil 1	3000
1:3 dil	1000
Serial dil	333
Dil 4	111

Table 6: Transcript Summary by Disease

Probe	Prostate Cancer	Breast Cancer	Colorectal Cancer	Melanoma Skin Cancer	Lung Cancer	Ovarian Cancer	Testicular Cancer
1	•					•	
2	•	•	•			•	•
3	•		•			•	•
4	•						•
5							
6				•	•	•	
7							
8			•		•		
9			•				
10			•	•	•		
11			•	•		•	•
12			•			•	•
13							•
14				•			
15				•		•	•
16				•			•
17							
20				•	•	•	•

Claims:

1. An isolated mitochondrial fusion transcript associated with cancer, wherein the transcript comprises a nucleic acid sequence as set forth in SEQ ID NO: 18 or SEQ ID NO: 33.
2. A mitochondrial fusion protein corresponding to the fusion transcript of claim 1 or a transcript having an amino acid sequence as set forth in SEQ ID NO: 34 or SEQ ID NO: 49.
3. An isolated mitochondrial DNA (mtDNA) encoding the fusion transcript of claim 1.
4. The isolated mtDNA of claim 3 having a nucleic acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 17.
5. A hybridization probe having a nucleic acid sequence complementary to the mitochondrial fusion transcript according to claim 1.
6. A hybridization probe having a nucleic acid sequence complementary to the mtDNA of claim 3 or 4.
7. A method of detecting a cancer in a mammal, the method comprising detecting in a tissue sample from the mammal the presence of the mitochondrial fusion transcript according to claim 1 or the presence of the mtDNA according to claim 3 or 4.
8. A method of detecting a cancer in a mammal, the method comprising assaying a tissue sample from the mammal for the presence of the mitochondrial fusion transcript according to claim 1 by hybridizing the sample with a hybridization probe having a nucleic acid sequence complementary to at least a portion of the mitochondrial fusion transcript.
9. A method of detecting a cancer in a mammal, the method comprising assaying a tissue sample from the mammal for the presence of the mtDNA according to claim 3 or 4 by hybridizing the sample with a hybridization probe having a nucleic acid sequence complementary to at least a portion of the mtDNA.

10. The method of claim 8, wherein said hybridization probe has a nucleic acid sequence complementary to a section of said mitochondrial fusion transcript comprising an expressed section of mtDNA comprising a junction point resulting from a deletion spanning nucleotides 8469 to 13447 of the human mtDNA genome.
11. The method of claim 9, wherein said hybridization probe has a nucleic acid sequence complementary to a section of said mtDNA comprising a junction point resulting from a deletion spanning nucleotides 8469 to 13447 of the human mtDNA genome.
12. The method of any one of claims 7 to 11, wherein the cancer is one or more of prostate cancer, testicular cancer, ovarian cancer, breast cancer, colorectal cancer, lung cancer, or melanoma skin cancer.
13. The method of any one of claims 7 to 11, wherein the cancer is prostate cancer or ovarian cancer.
14. The method of any one of claims 8 to 13, wherein the assay comprises:
 - a) conducting a hybridization reaction to allow said probe to hybridize to the mitochondrial fusion transcript or mtDNA, respectively;
 - b) quantifying the amount of the mitochondrial fusion transcript or mtDNA in said sample by quantifying the amount of said transcript or mtDNA hybridized to said probe; and,
 - c) comparing the amount of the mitochondrial fusion transcript or mtDNA in the sample to at least one known reference value.
15. The method of claim 14, wherein the assay is carried out using diagnostic imaging technology, branched DNA technology, or PCR.
16. The method of claim 15, wherein the diagnostic imaging technology comprises high throughput microarray analysis.
17. A kit for conducting an assay for detecting the presence of a cancer in a mammal, said kit comprising at least one hybridization probe complementary to the fusion transcript of claim 1.

18. A kit for conducting an assay for detecting the presence of a cancer in a mammal, said kit comprising at least one hybridization probe complementary to the mtDNA of claim 3 or 4.
19. A screening tool comprised of a microarray having 10's, 100's, or 1000's of mitochondrial fusion transcripts according to claim 1 for identification of those transcripts associated with cancer.
20. A screening tool comprised of a microarray having 10's, 100's, or 1000's of mitochondrial DNAs (mtDNAs) according to claim 3 or 4 for identification of those mtDNAs associated with cancer.
21. A screening tool comprised of a multiplexed branched DNA assay having 10's, 100's, or 1000's of mitochondrial fusion transcripts according to claim 1 for identification of those transcripts associated with cancer.
22. A screening tool comprised of a multiplexed branched DNA assay having 10's, 100's, or 1000's of mitochondrial DNAs (mtDNAs) according to claim 3 or 4 for identification of those mtDNAs associated with cancer.

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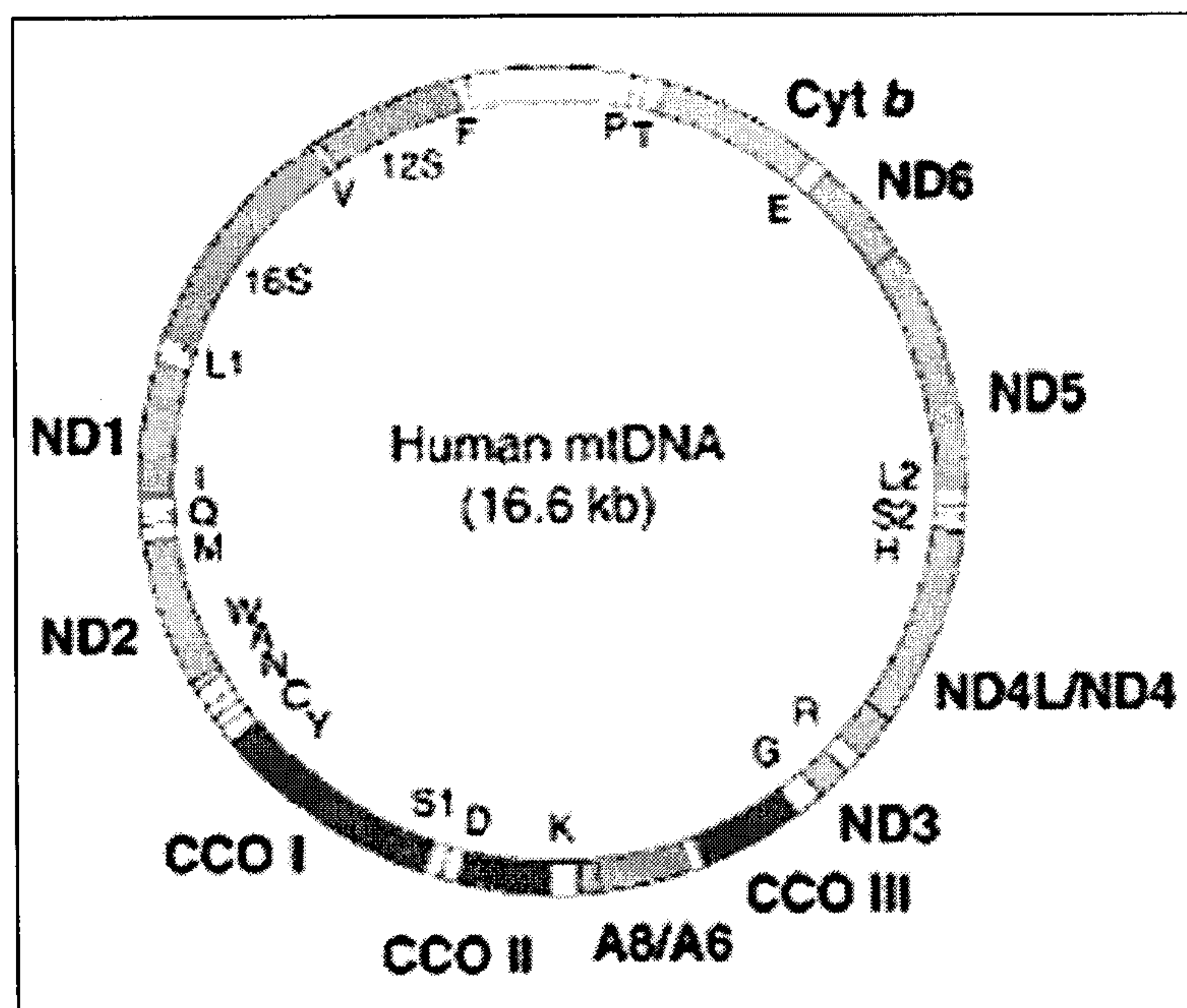


Figure 1

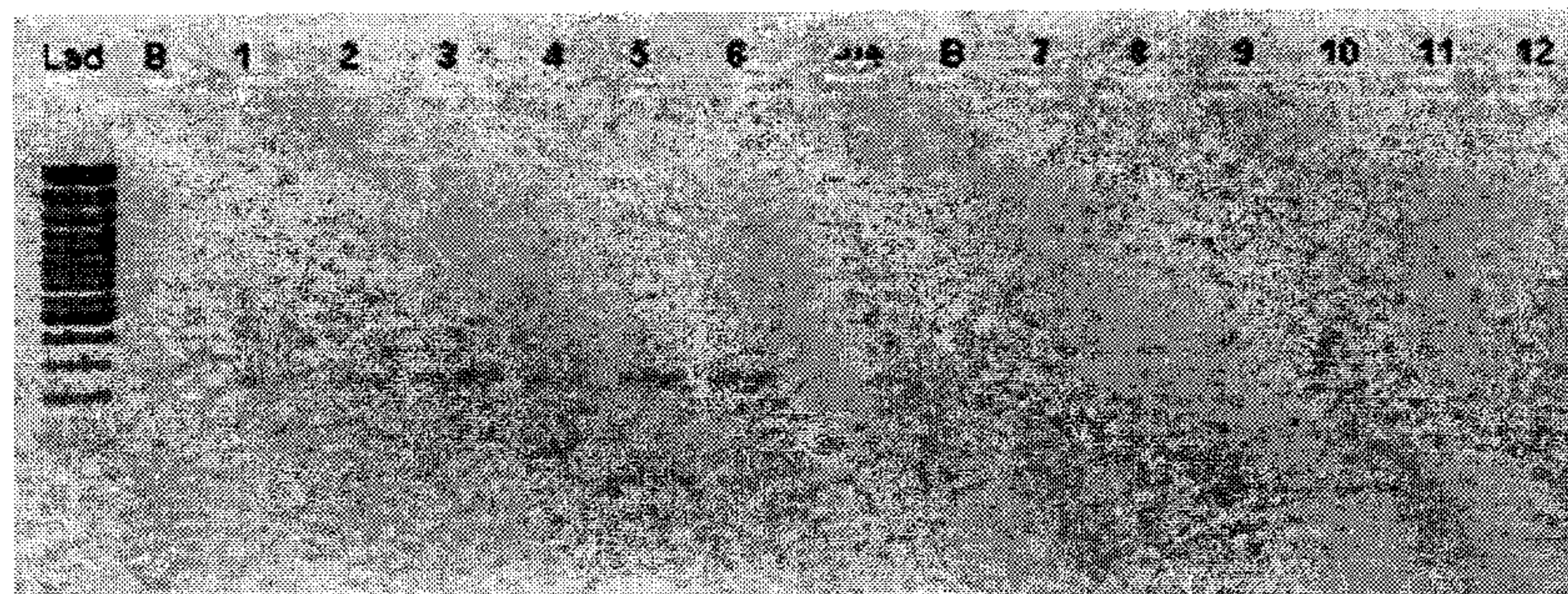


Figure 2

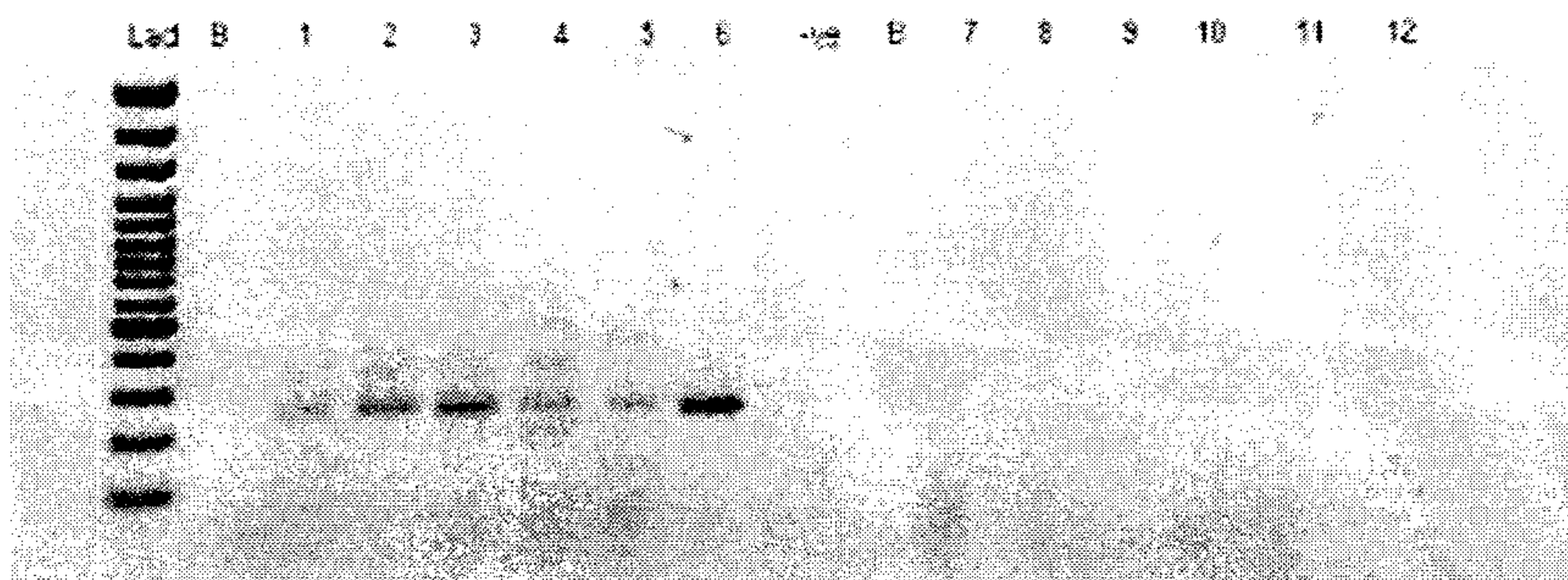


Figure 3

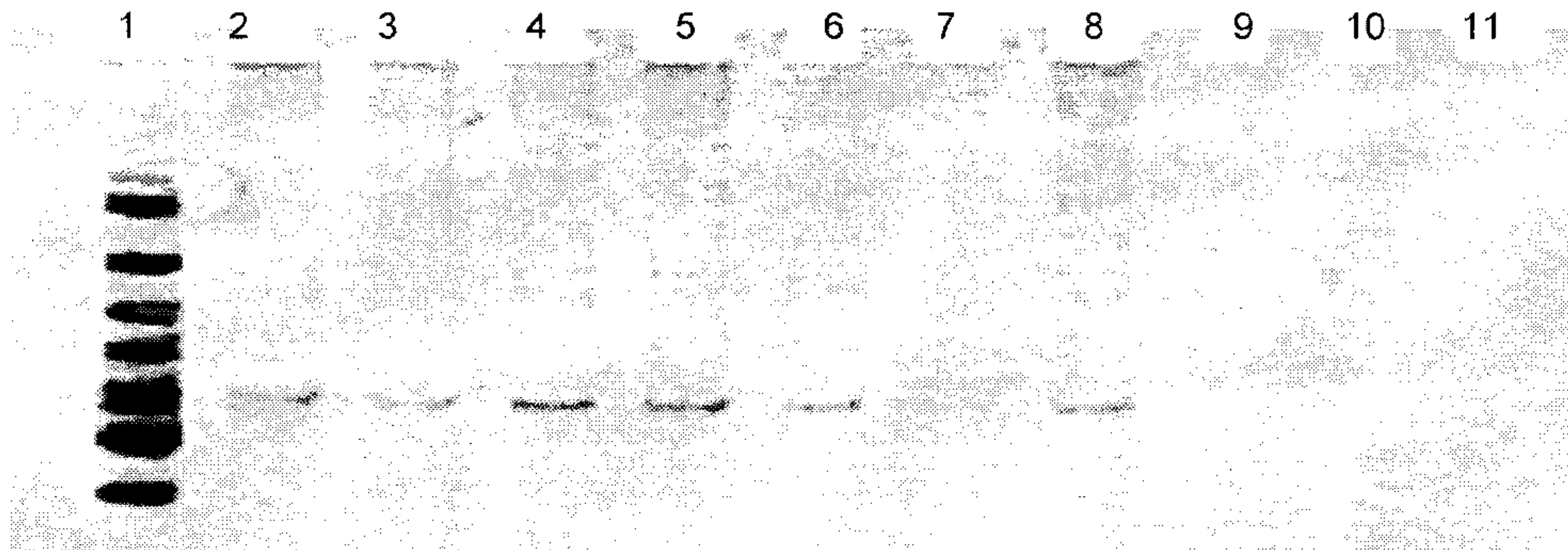
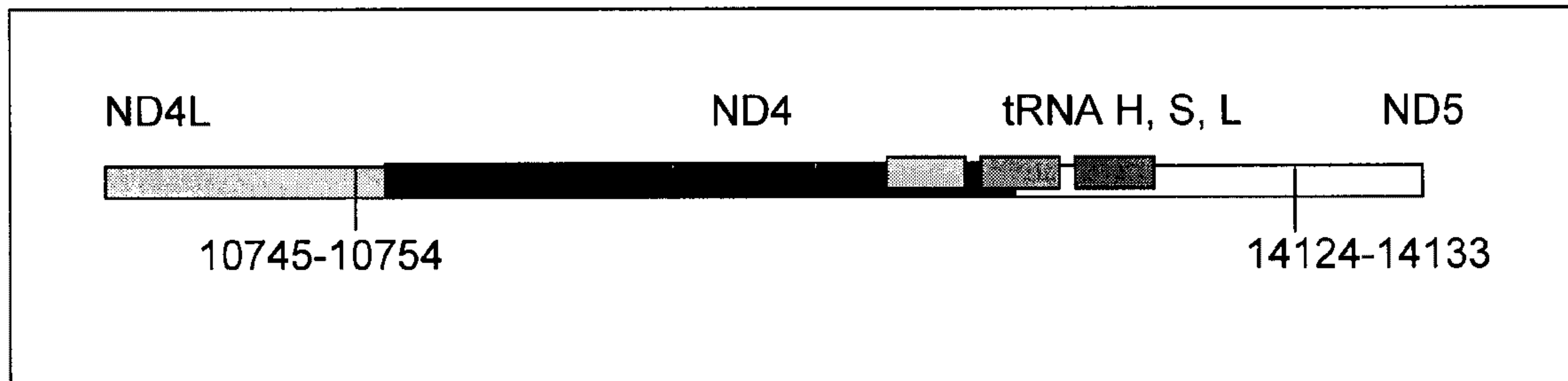
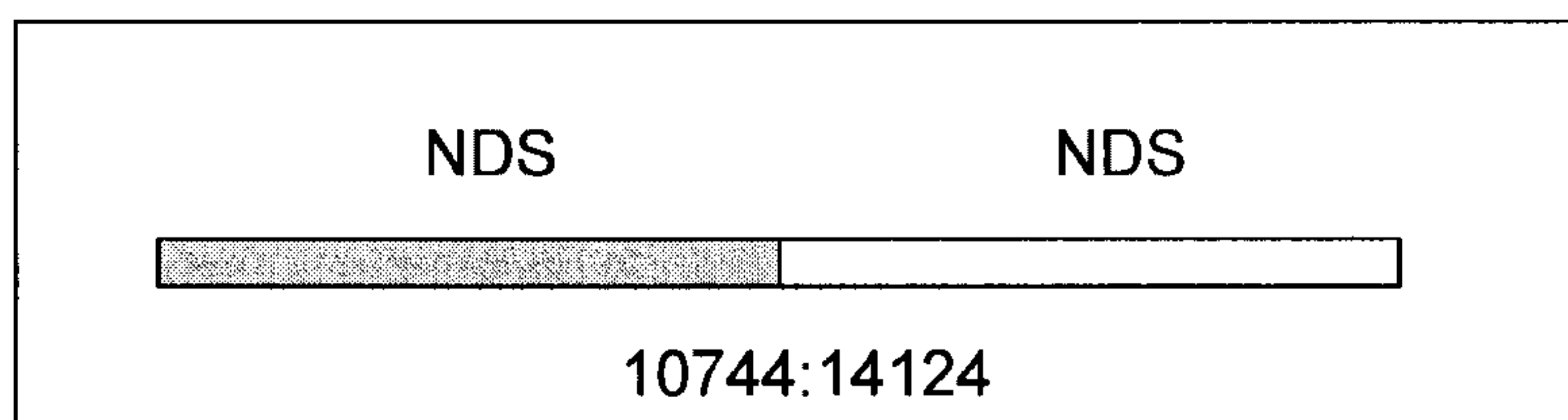
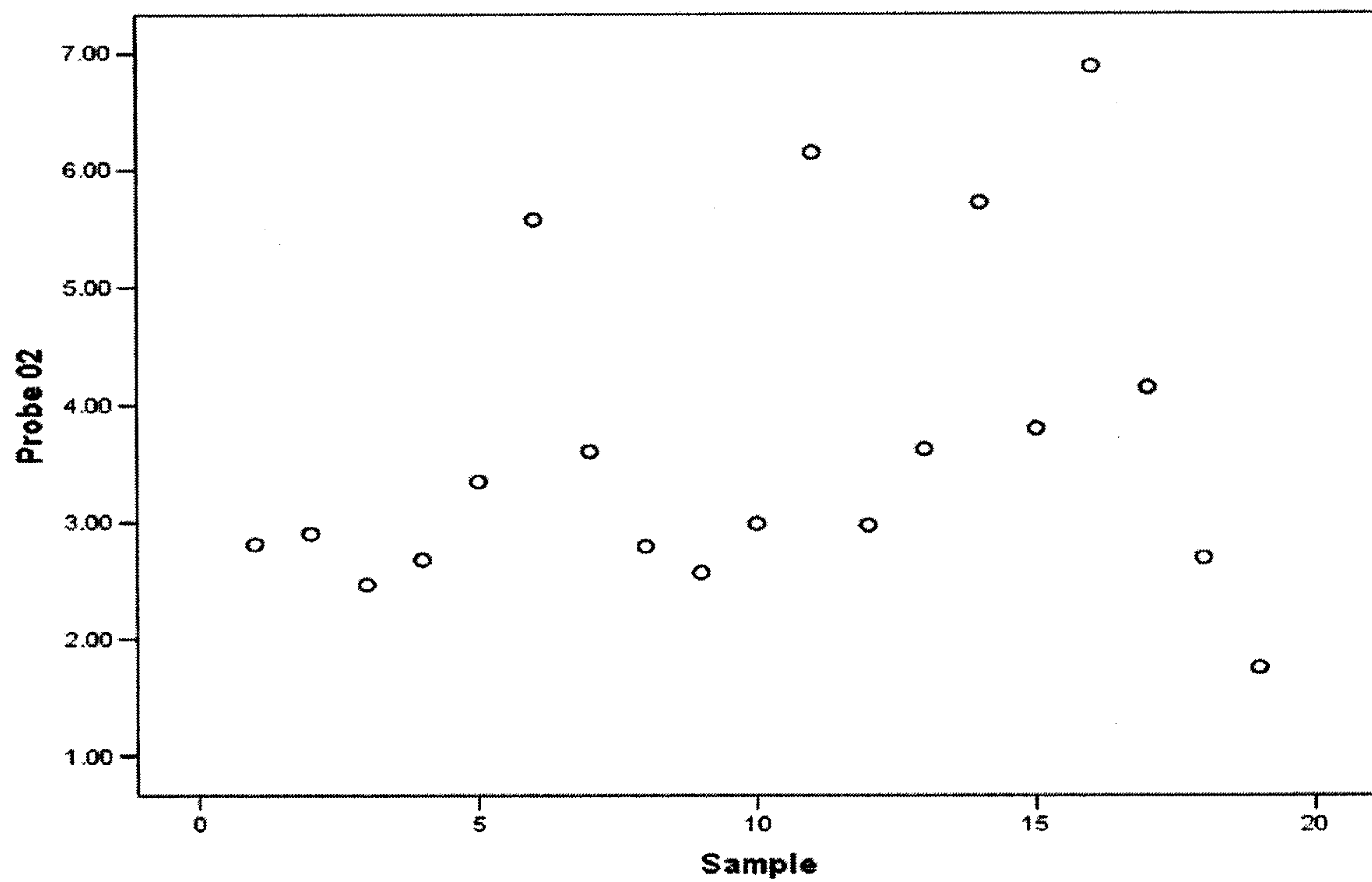


Figure 4

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**Figure 5a****Figure 5b**

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ROC Curve

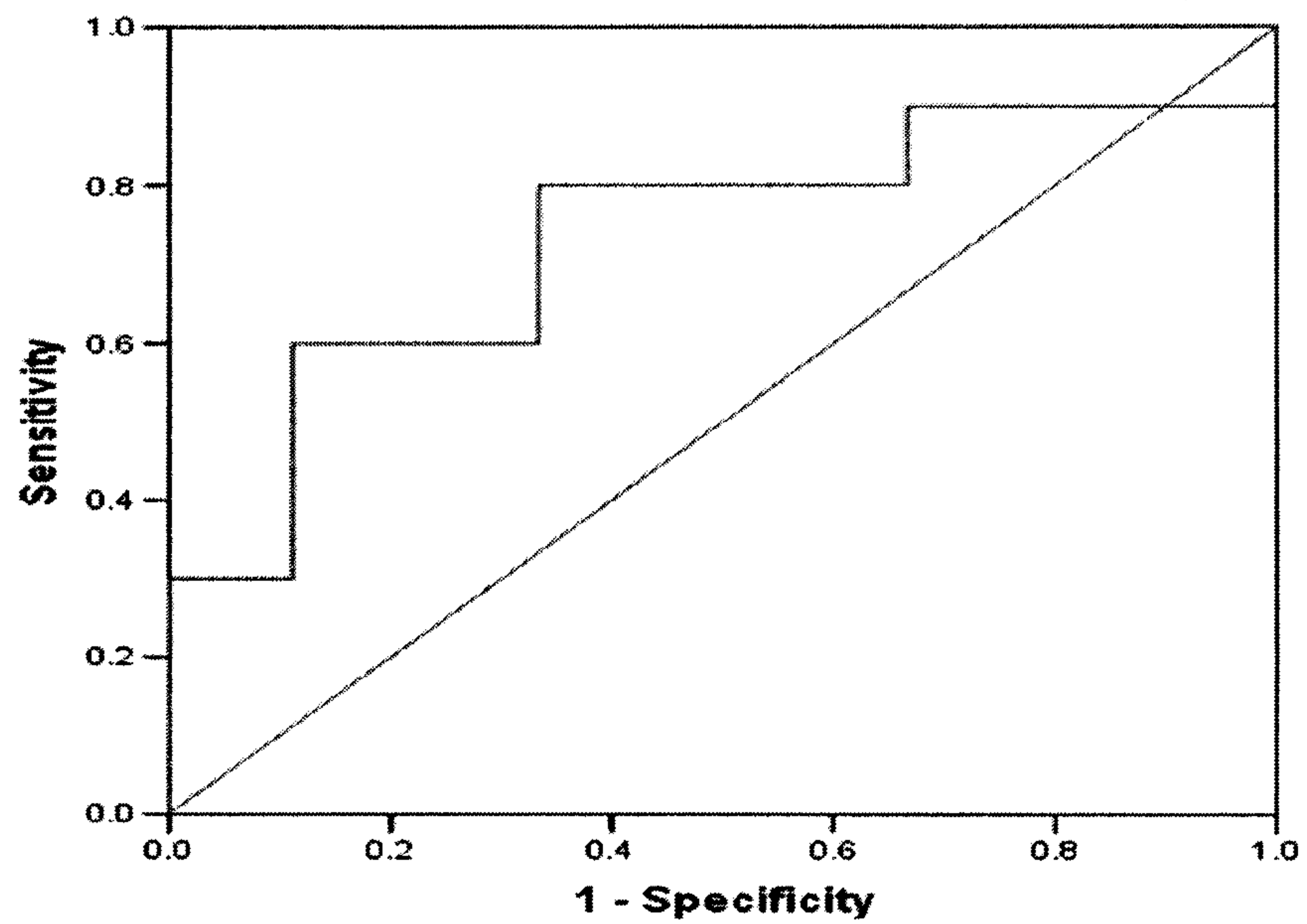


Figure 6a

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Coordinates of the Curve

Test Result Variable(s): Probe 02

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
.7568	1.000	1.000
2.1107	.900	1.000
2.5160	.900	.889
2.6222	.900	.778
2.6862	.900	.667
2.7433	.800	.667
2.8012	.800	.556
2.8558	.800	.444
2.9369	.800	.333
2.9800	.700	.333
3.1676	.600	.333
3.4764	.600	.222
3.6129	.600	.111
3.7104	.500	.111
3.9727	.400	.111
4.8624	.300	.111
5.6516	.300	.000
5.9374	.200	.000
6.5163	.100	.000
7.8827	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Probe 02

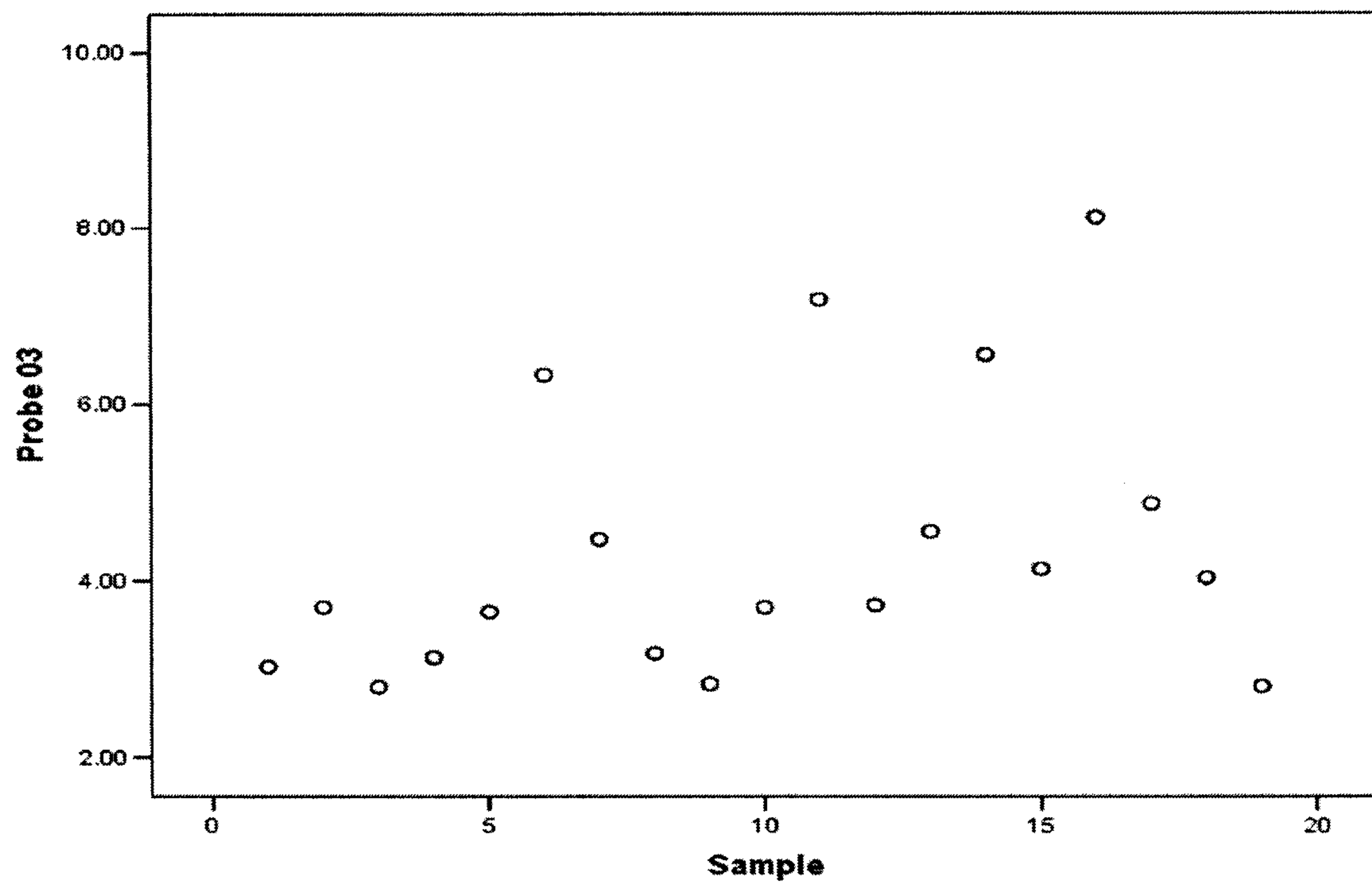
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.733	.121	.086	.497	.970

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 6a (cond.)

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ROC Curve

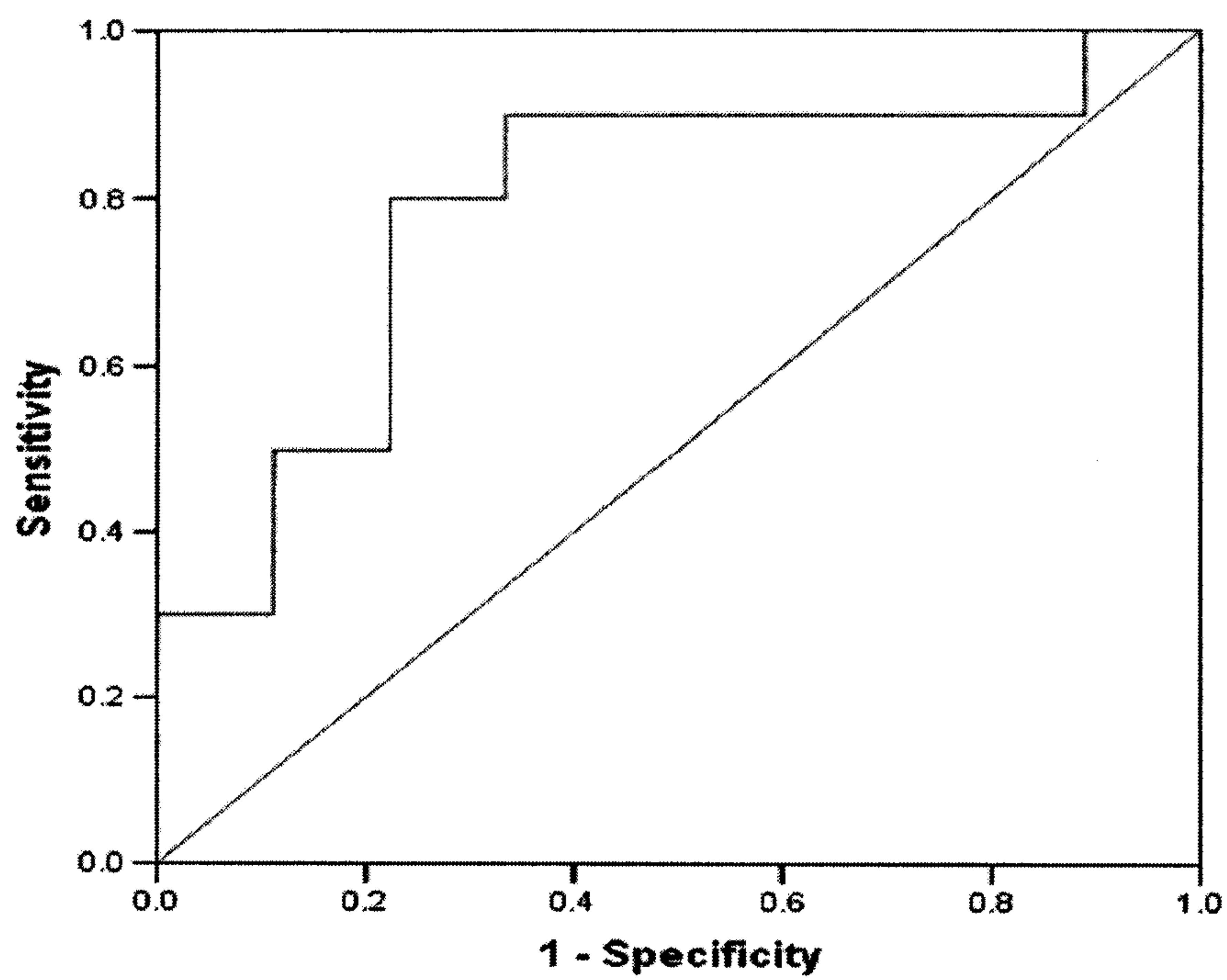


Figure 6b

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Coordinates of the Curve

Test Result Variable(s): Probe 03

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
1.7966	1.000	1.000
2.7983	1.000	.889
2.8154	.900	.889
2.9284	.900	.778
3.0788	.900	.667
3.1540	.900	.556
3.4096	.900	.444
3.6685	.900	.333
3.6959	.800	.333
3.7094	.800	.222
3.8759	.700	.222
4.0813	.600	.222
4.3005	.500	.222
4.5109	.500	.111
4.7108	.400	.111
5.6001	.300	.111
6.4437	.300	.000
6.8705	.200	.000
7.6506	.100	.000
9.1160	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Probe 03

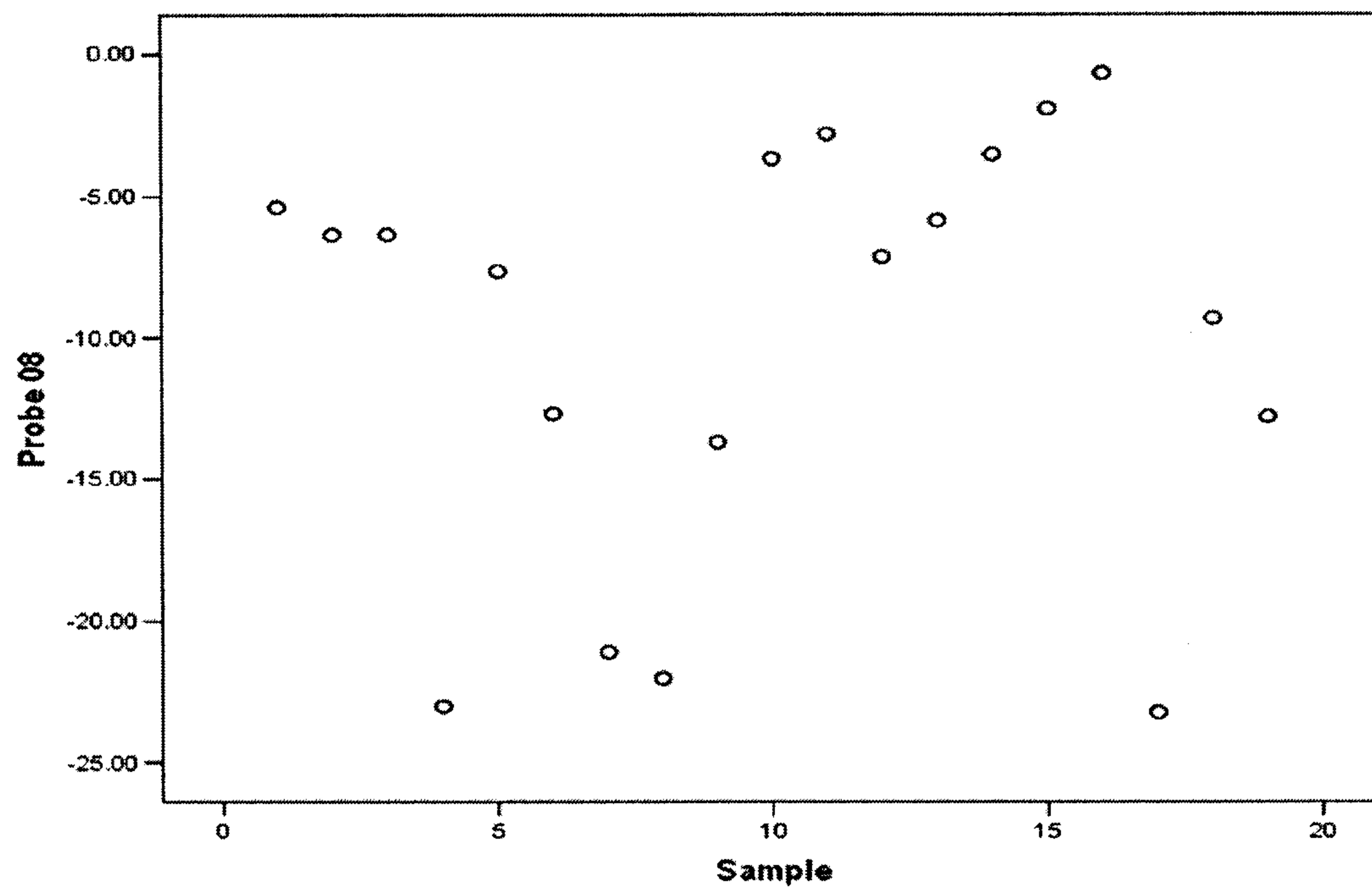
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.789	.110	.034	.572	1.005

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 6b (cond.)

9/133



ROC Curve

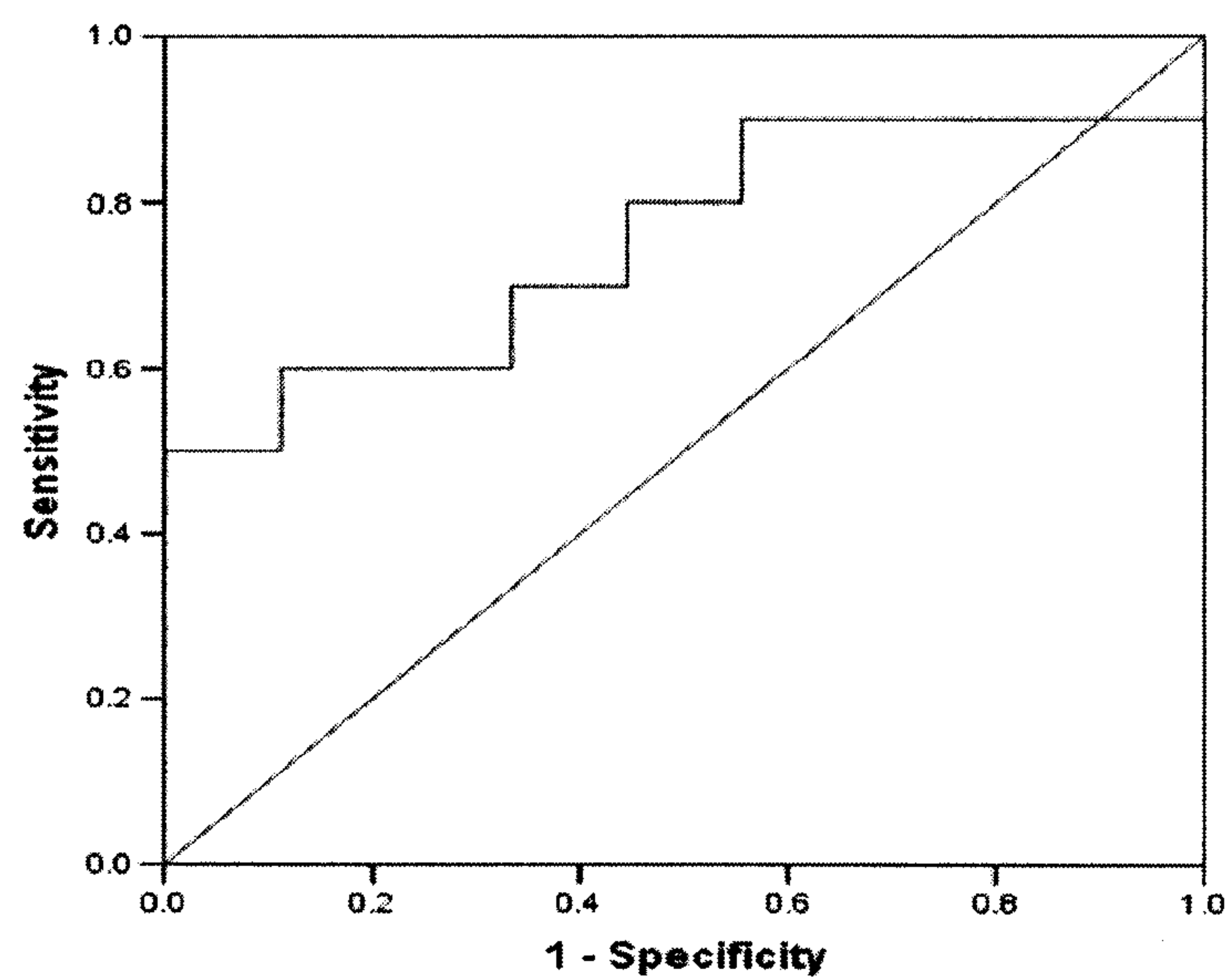


Figure 6c

10/133

Coordinates of the Curve

Test Result Variable(s): Probe 08

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-24.2356	1.000	1.000
-23.1238	.900	1.000
-22.5180	.900	.889
-21.5598	.900	.778
-17.4046	.900	.667
-13.2542	.900	.556
-12.7454	.800	.556
-11.0063	.800	.444
-8.4803	.700	.444
-7.3886	.700	.333
-6.7456	.600	.333
-6.3510	.600	.222
-6.0975	.600	.111
-5.6176	.500	.111
-4.5241	.500	.000
-3.5933	.400	.000
-3.1617	.300	.000
-2.3596	.200	.000
-1.3003	.100	.000
.3178	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

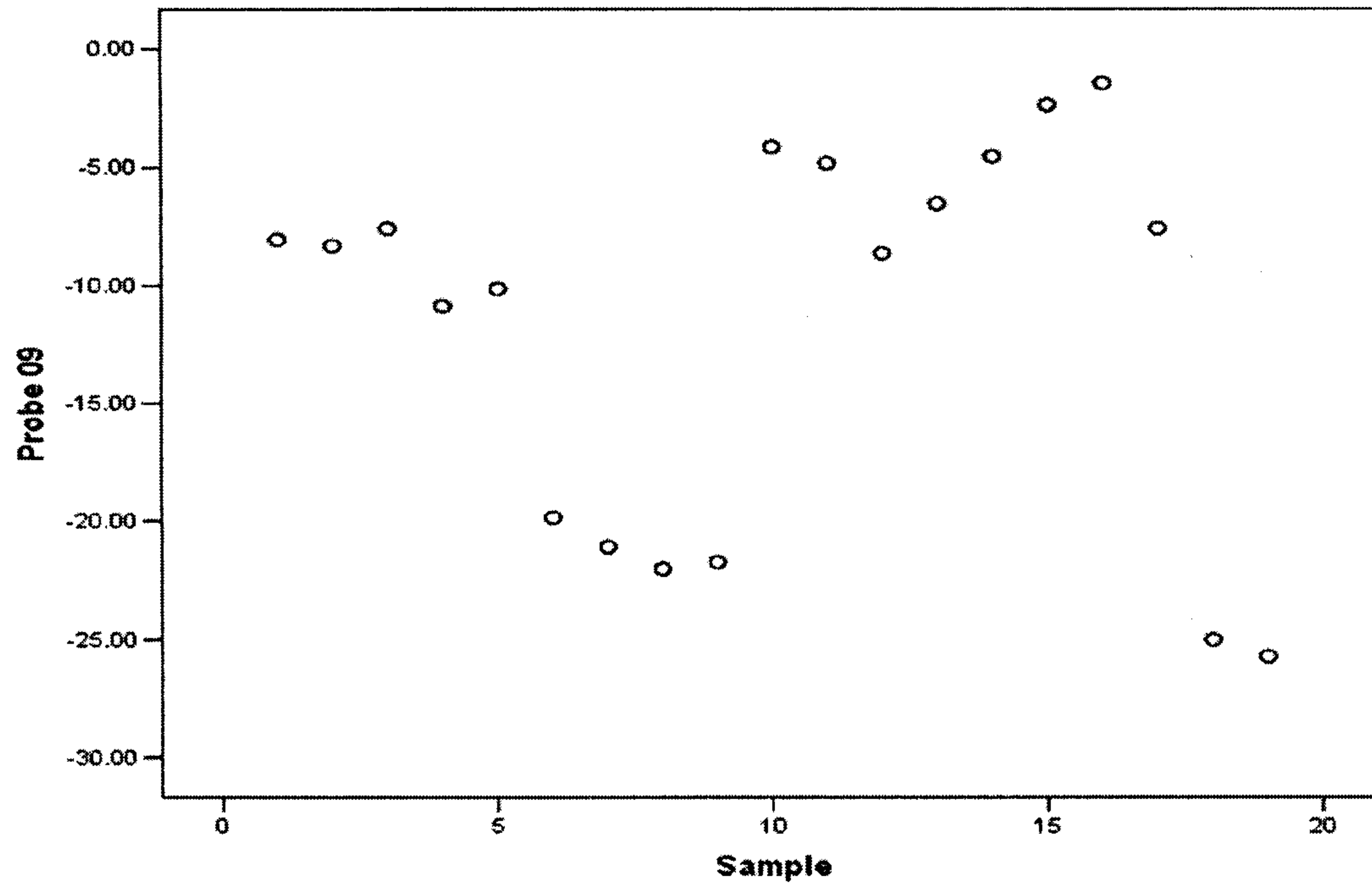
Test Result Variable(s): Probe 08

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.756	.116	.060	.528	.983

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 6c (cond.)

11/133



ROC Curve

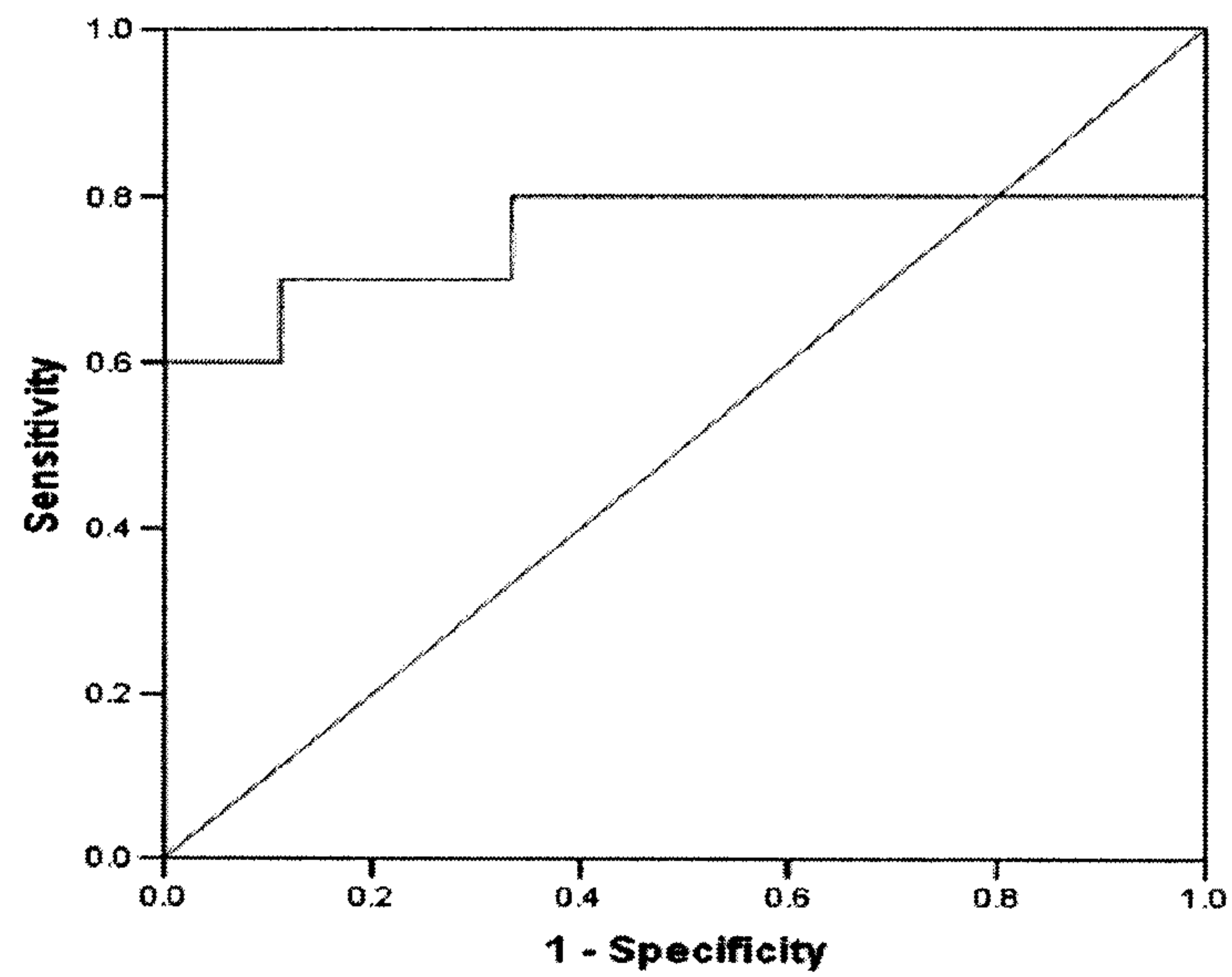


Figure 6d

12/133

Coordinates of the Curve

Test Result Variable(s): Probe 09

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-26.7218	1.000	1.000
-25.3641	.900	1.000
-23.5151	.800	1.000
-21.8829	.800	.889
-21.4187	.800	.778
-20.4804	.800	.667
-15.3686	.800	.556
-10.5043	.800	.444
-9.3795	.800	.333
-8.4552	.700	.333
-8.1579	.700	.222
-7.7931	.700	.111
-7.5555	.600	.111
-7.0324	.600	.000
-5.6631	.500	.000
-4.6705	.400	.000
-4.3237	.300	.000
-3.2382	.200	.000
-1.8956	.100	.000
-.4350	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Probe 09

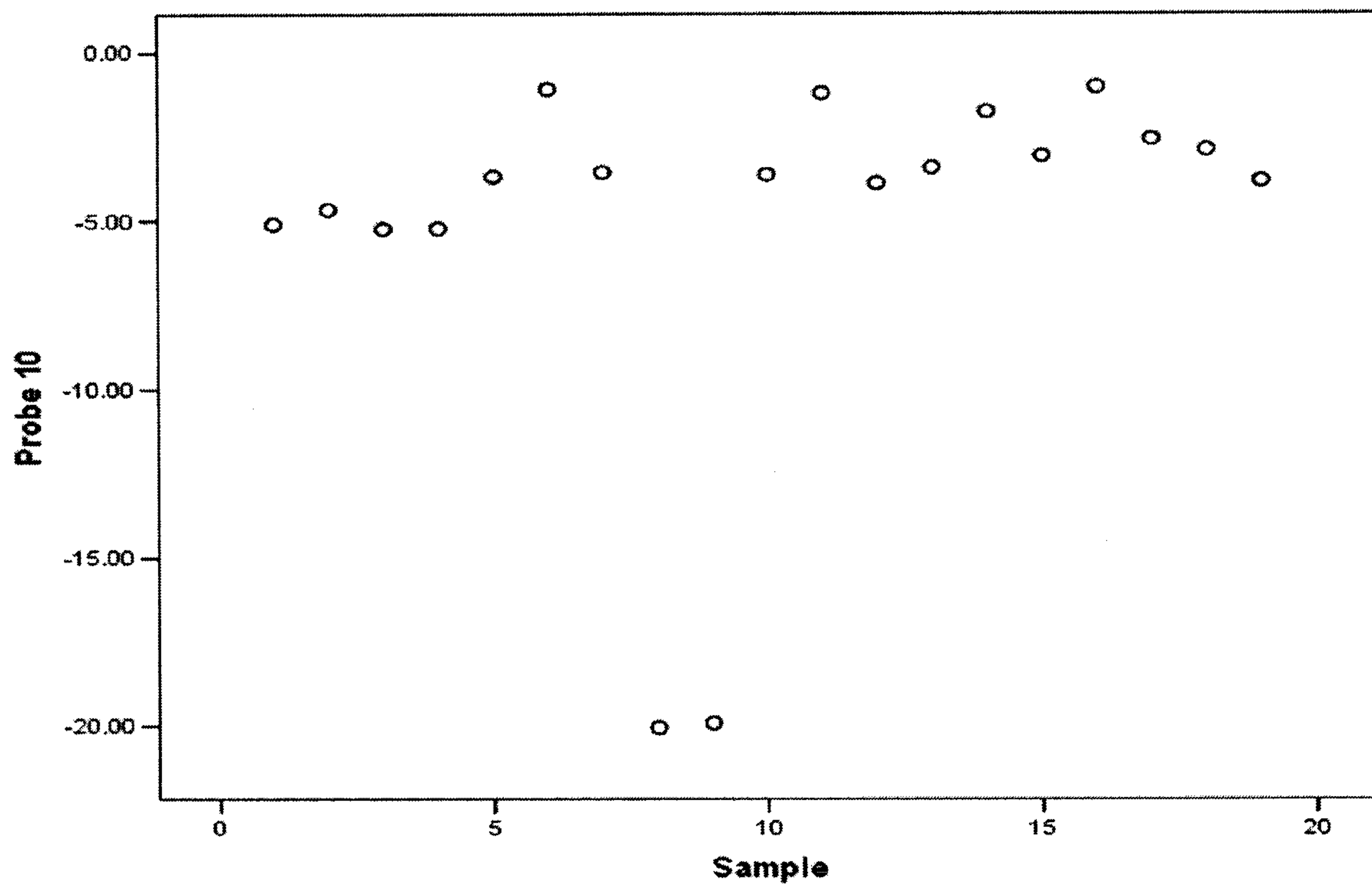
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.756	.127	.060	.507	1.004

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 6d (cond.)

13/133



ROC Curve

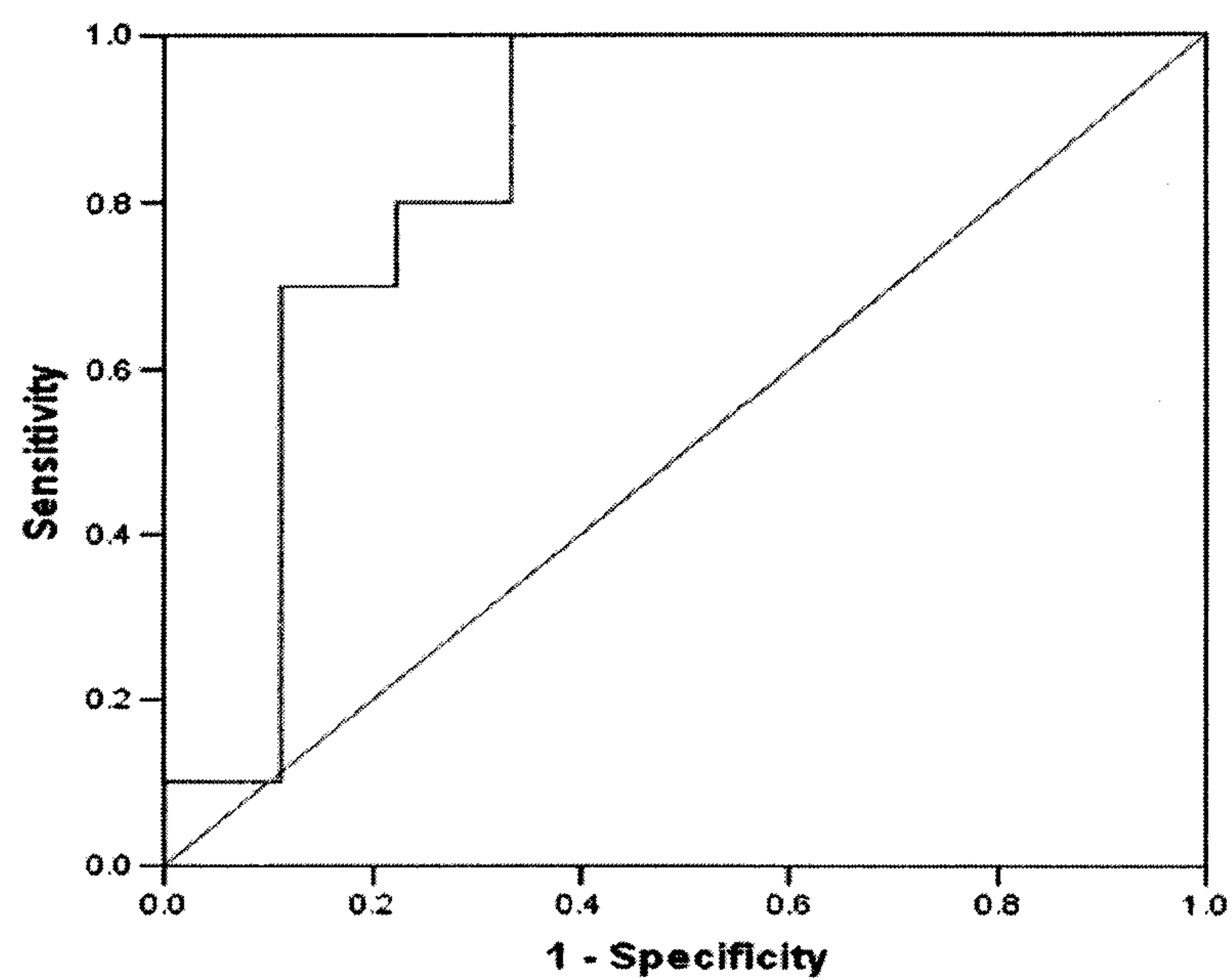


Figure 6e

14/133

Coordinates of the Curve

Test Result Variable(s): Probe 10

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-21.0353	1.000	1.000
-19.9698	1.000	.889
-12.5697	1.000	.778
-5.2295	1.000	.667
-5.1624	1.000	.556
-4.8838	1.000	.444
-4.2646	1.000	.333
-3.8272	.900	.333
-3.7343	.800	.333
-3.6478	.800	.222
-3.5841	.700	.222
-3.4781	.700	.111
-3.2304	.600	.111
-2.9591	.500	.111
-2.7091	.400	.111
-2.1473	.300	.111
-1.4695	.200	.111
-1.1392	.100	.111
-1.0450	.100	.000
-.0097	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

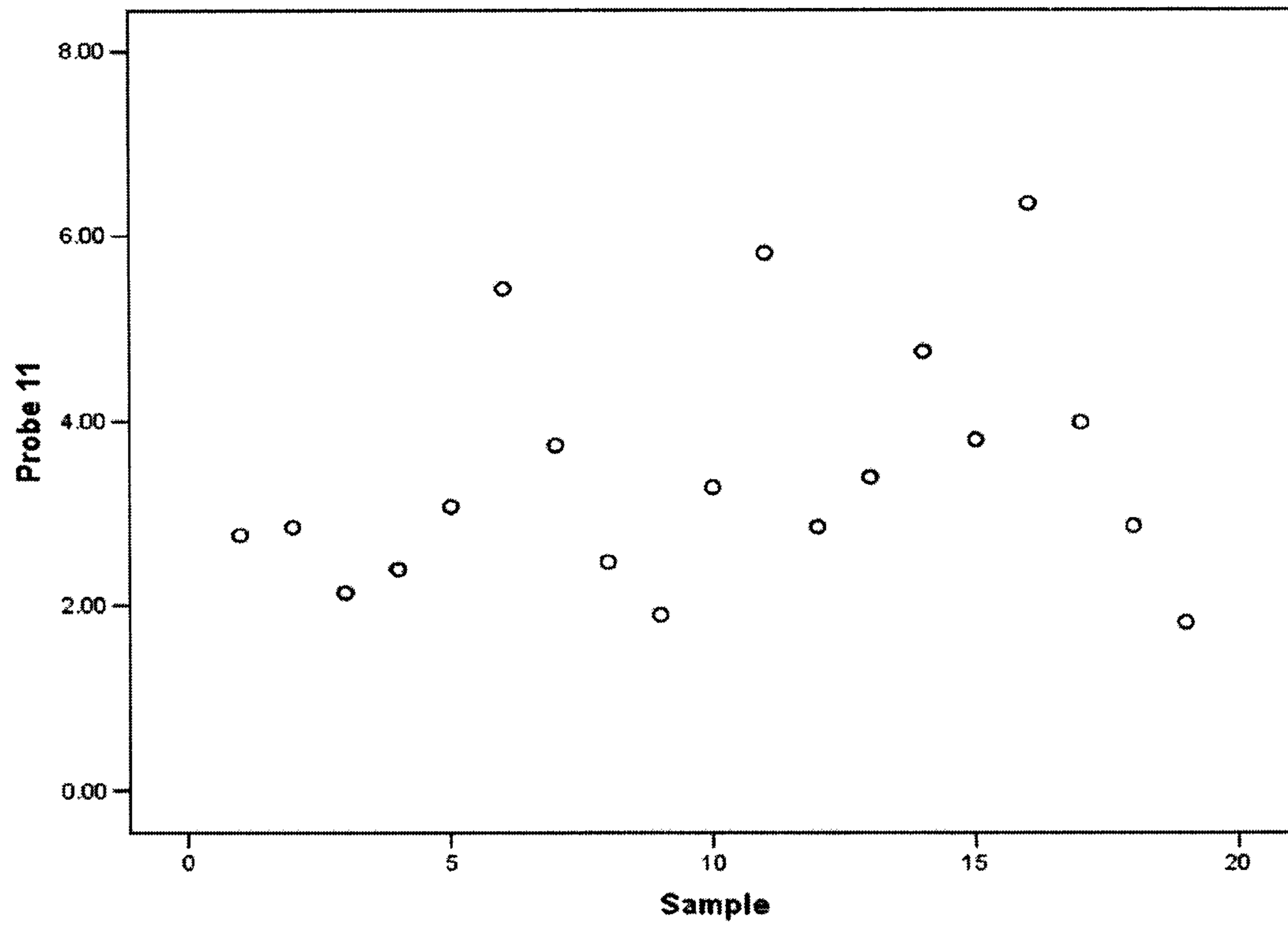
Test Result Variable(s): Probe 10

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.844	.102	.011	.645	1.044

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 6e (cond.)

15/133



ROC Curve

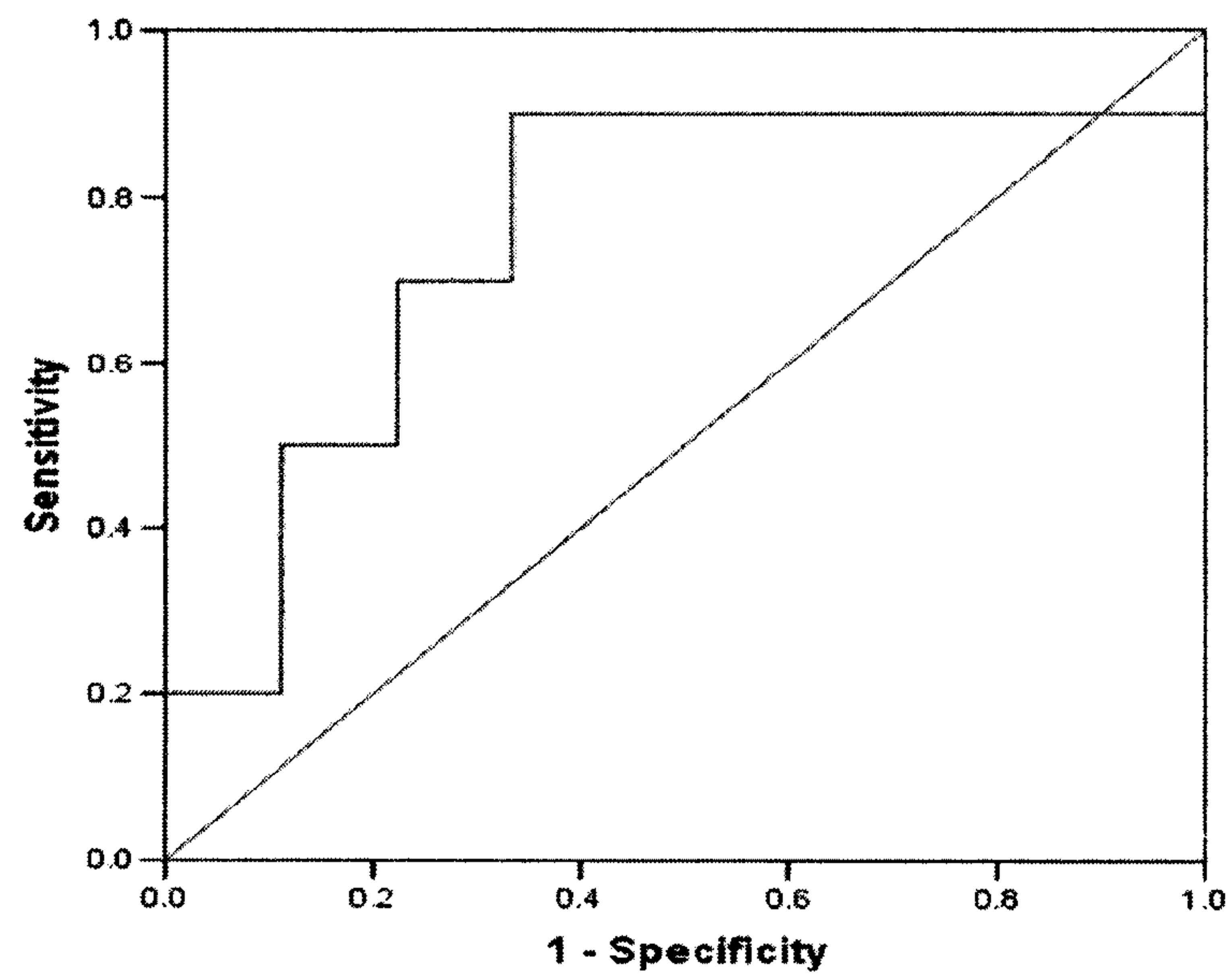


Figure 6f

16/133

Coordinates of the Curve

Test Result Variable(s): Probe 11

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
.8199	1.000	1.000
1.8582	.900	1.000
2.0162	.900	.889
2.2632	.900	.778
2.4301	.900	.667
2.6165	.900	.556
2.8041	.900	.444
2.8463	.900	.333
2.8543	.800	.333
2.9662	.700	.333
3.1753	.700	.222
3.3334	.600	.222
3.5634	.500	.222
3.7674	.500	.111
3.8907	.400	.111
4.3690	.300	.111
5.0928	.200	.111
5.6250	.200	.000
6.0840	.100	.000
7.3513	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

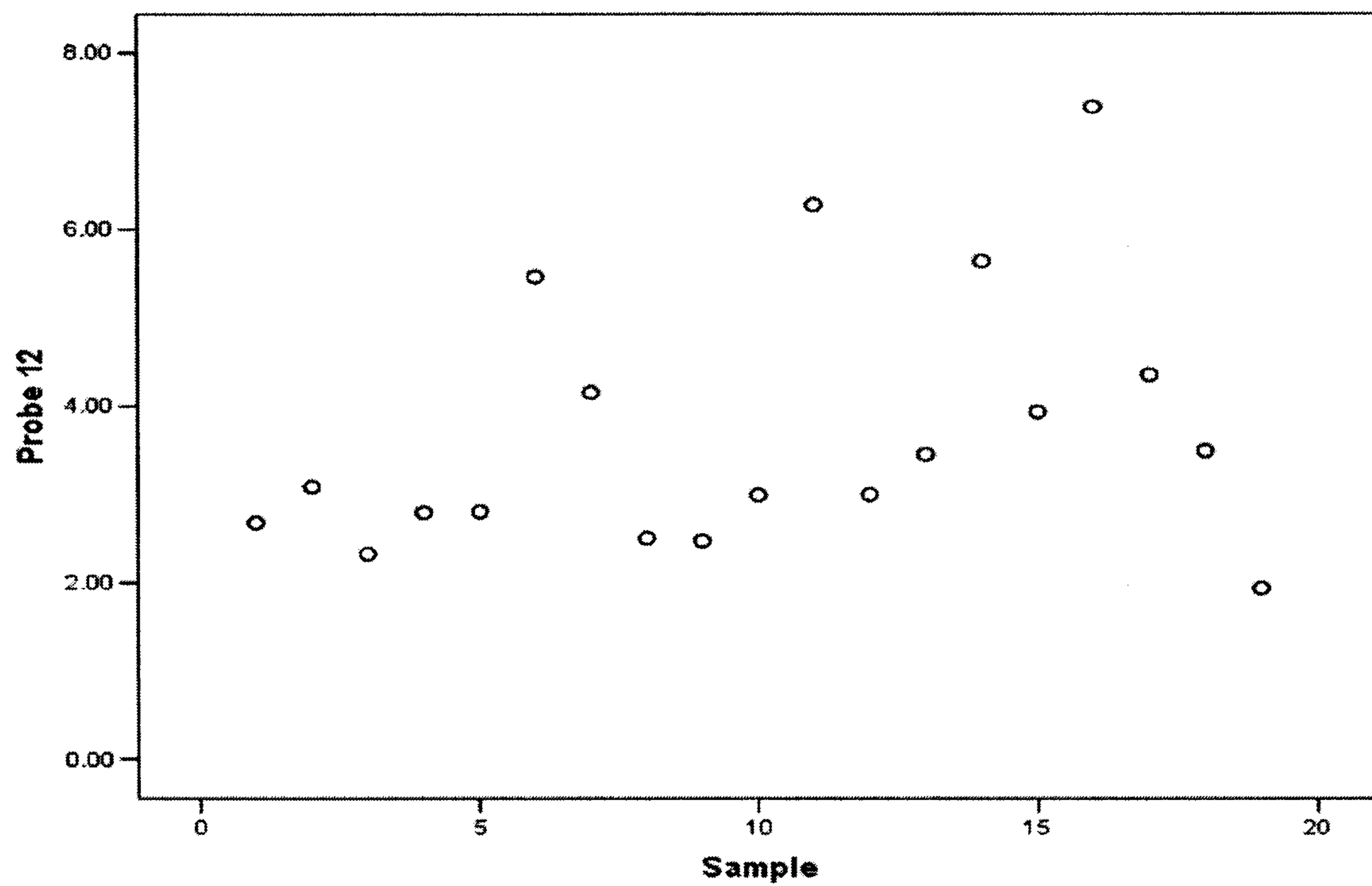
Test Result Variable(s): Probe 11

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.756	.120	.060	.520	.991

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 6f (cond.)

17/133



ROC Curve

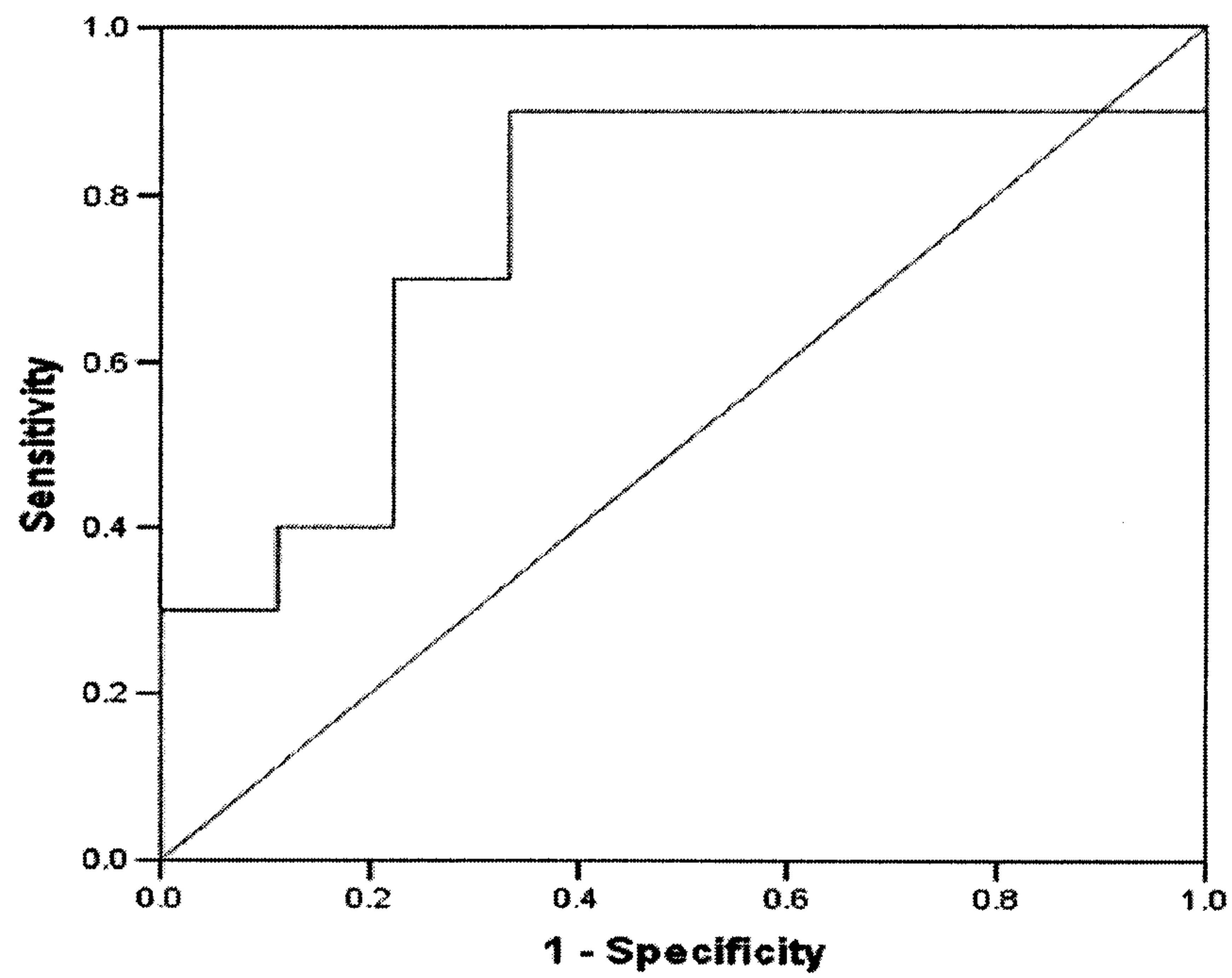


Figure 6g

18/133

Coordinates of the Curve

Test Result Variable(s): Probe 12

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
.9262	1.000	1.000
2.1243	.900	1.000
2.3943	.900	.889
2.4824	.900	.778
2.5862	.900	.667
2.7316	.900	.556
2.7936	.900	.444
2.8907	.900	.333
2.9855	.800	.333
3.0347	.700	.333
3.2626	.700	.222
3.4645	.600	.222
3.7076	.500	.222
4.0397	.400	.222
4.2497	.400	.111
4.9044	.300	.111
5.5481	.300	.000
5.9577	.200	.000
6.8291	.100	.000
8.3796	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Probe 12

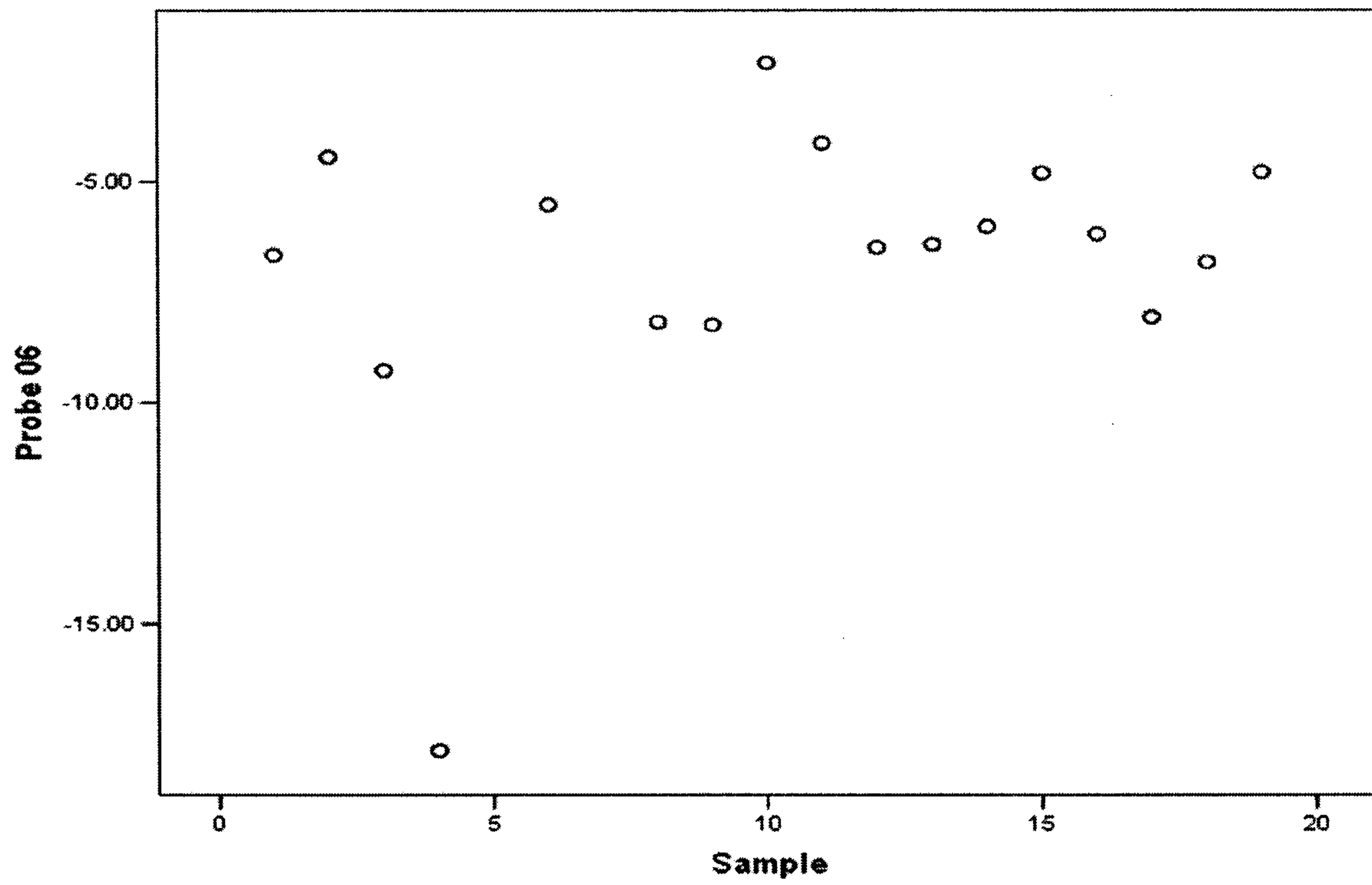
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.756	.119	.060	.522	.989

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 6g (cond.)

19/133



ROC Curve

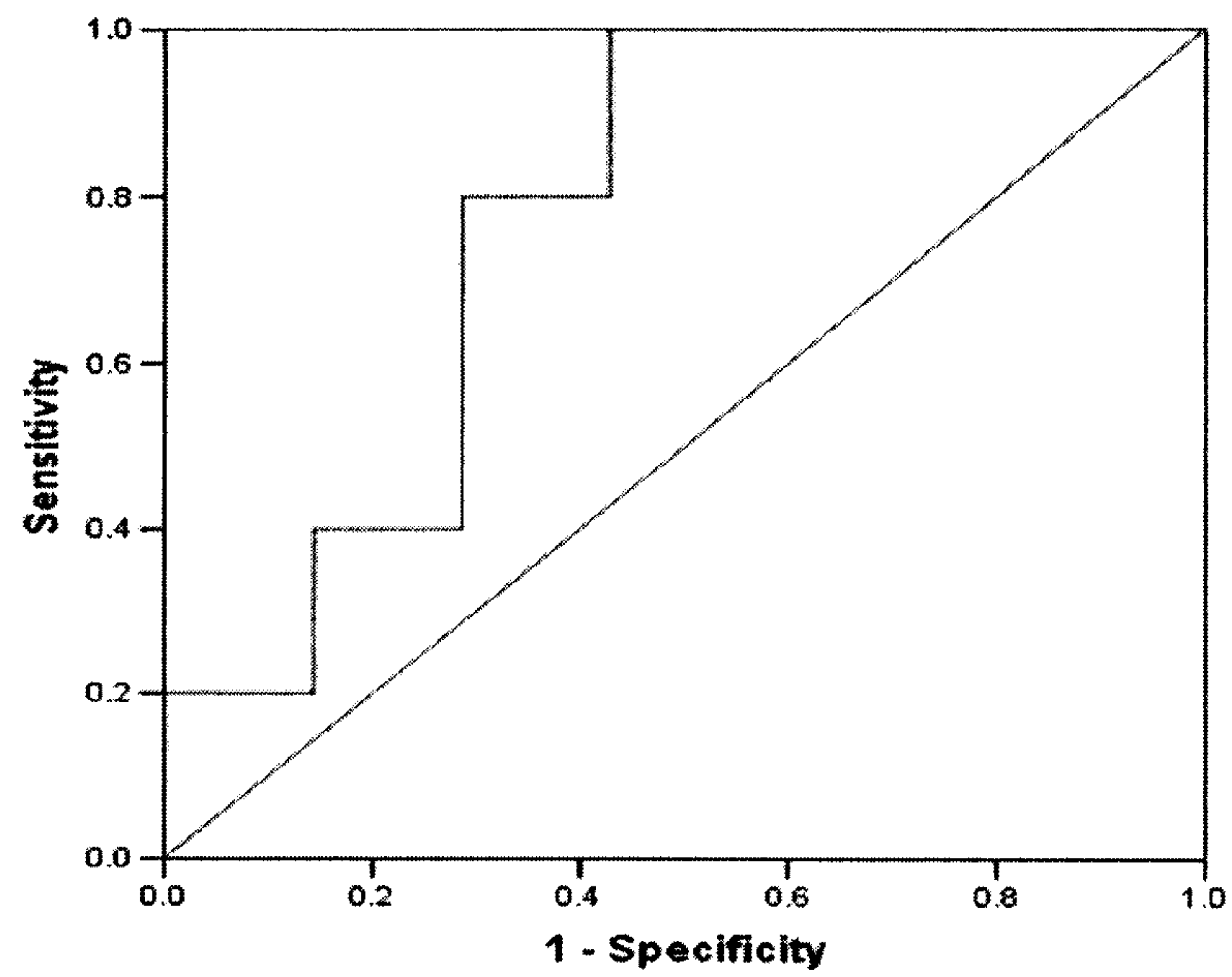


Figure 7a

20/133

Coordinates of the Curve

Test Result Variable(s): Probe 06

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-18.8885	1.000	1.000
-13.5845	1.000	.857
-8.7622	1.000	.714
-8.2117	1.000	.571
-8.1245	1.000	.429
-7.4446	.900	.429
-6.7388	.800	.429
-6.5691	.800	.286
-6.4475	.700	.286
-6.2968	.600	.286
-6.0956	.500	.286
-5.7666	.400	.286
-5.1640	.400	.143
-4.7948	.300	.143
-4.6092	.200	.143
-4.2851	.200	.000
-3.2263	.100	.000
-1.3185	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

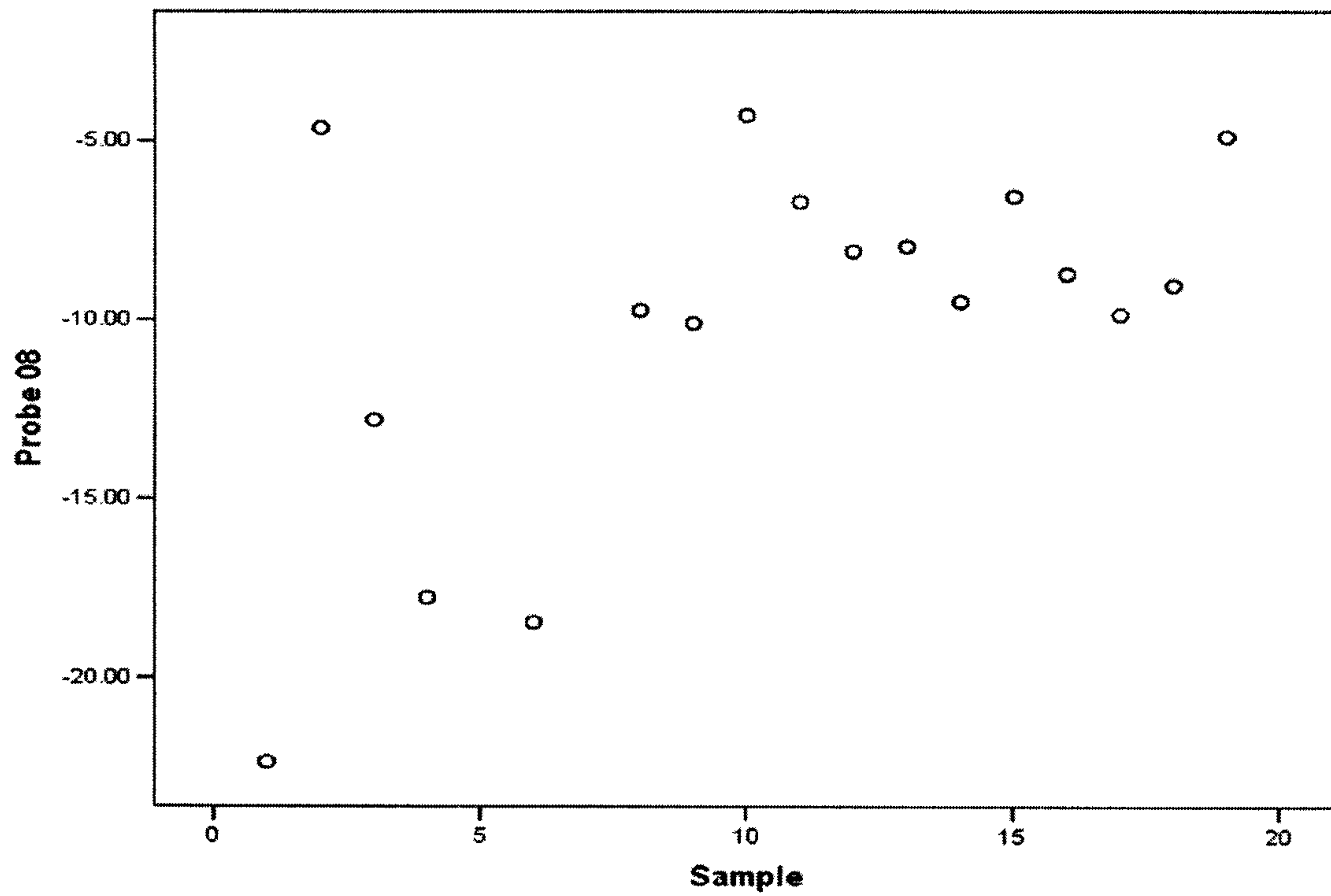
Test Result Variable(s): Probe 06

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.771	.129	.064	.518	1.025

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 7a (cond.)

21/133



ROC Curve

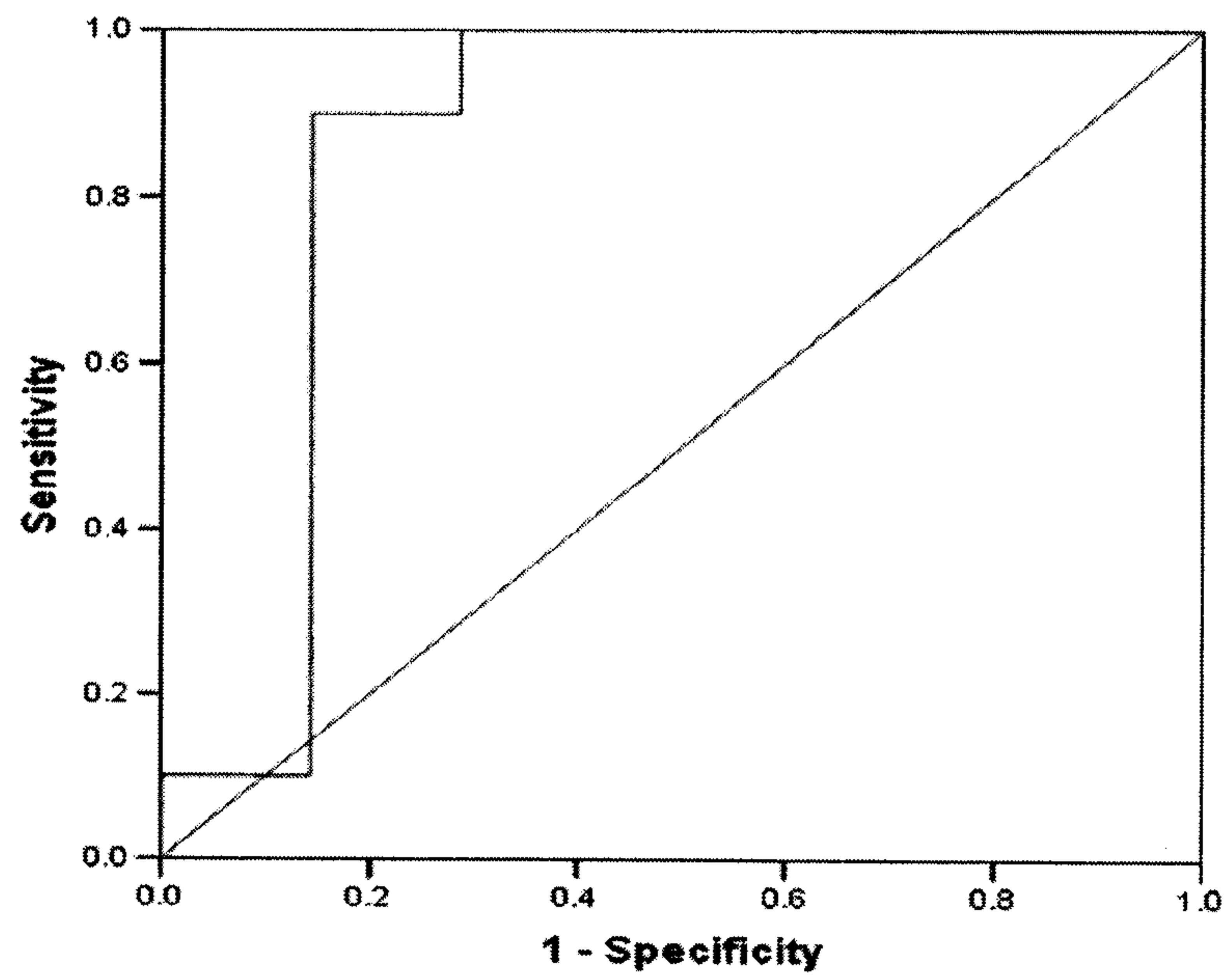


Figure 7b

22/133

Coordinates of the Curve

Test Result Variable(s): Probe 08

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-23.3755	1.000	1.000
-20.4163	1.000	.857
-18.1143	1.000	.714
-15.2935	1.000	.571
-11.4591	1.000	.429
-9.9810	1.000	.286
-9.8002	.900	.286
-9.6166	.900	.143
-9.2691	.800	.143
-8.8850	.700	.143
-8.4042	.600	.143
-8.0223	.500	.143
-7.3333	.400	.143
-6.6295	.300	.143
-5.7177	.200	.143
-4.7619	.100	.143
-4.4650	.100	.000
-3.2895	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

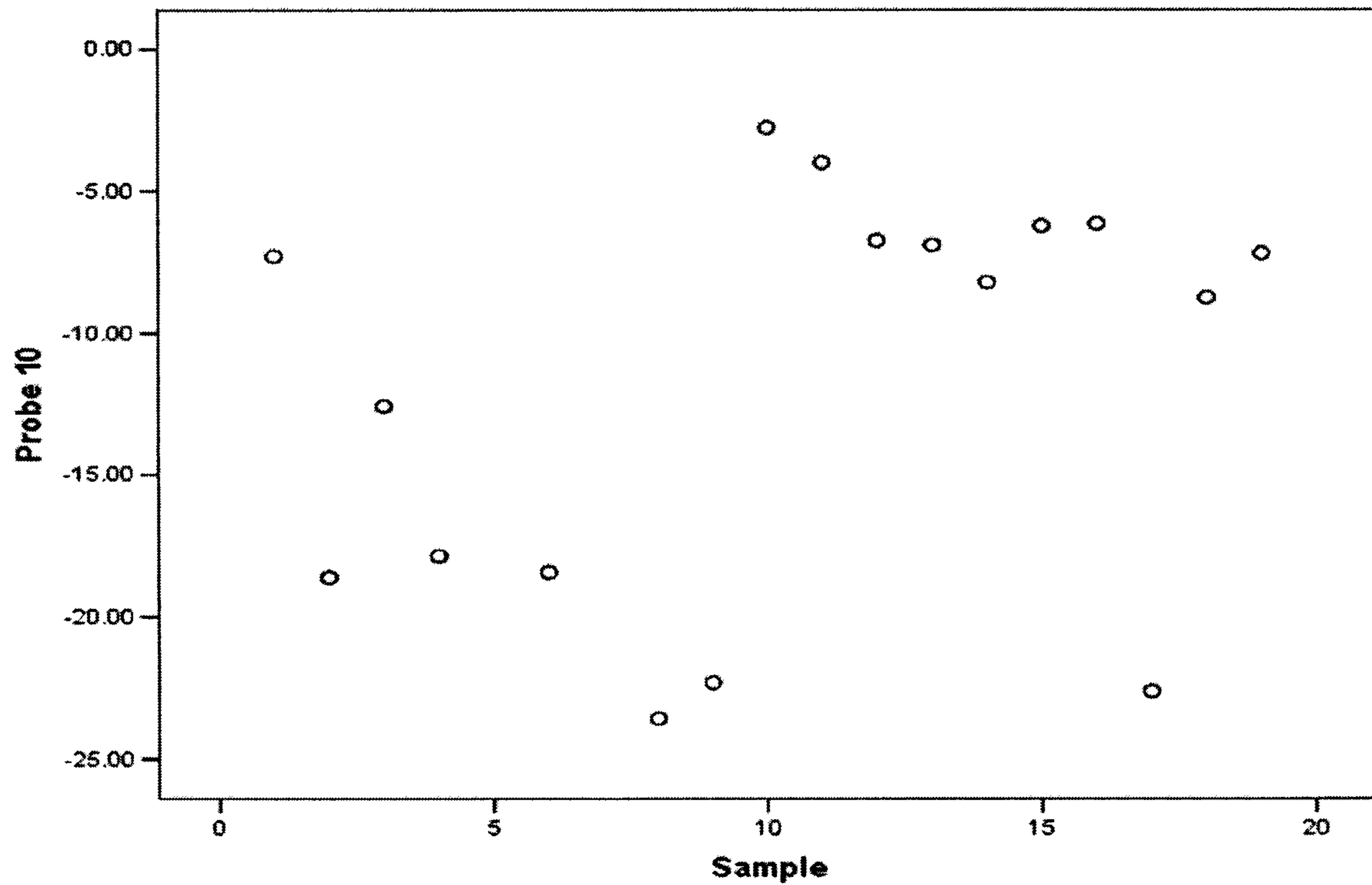
Test Result Variable(s): Probe 08

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.857	.121	.015	.621	1.093

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 7b (cond.)

23/133



ROC Curve

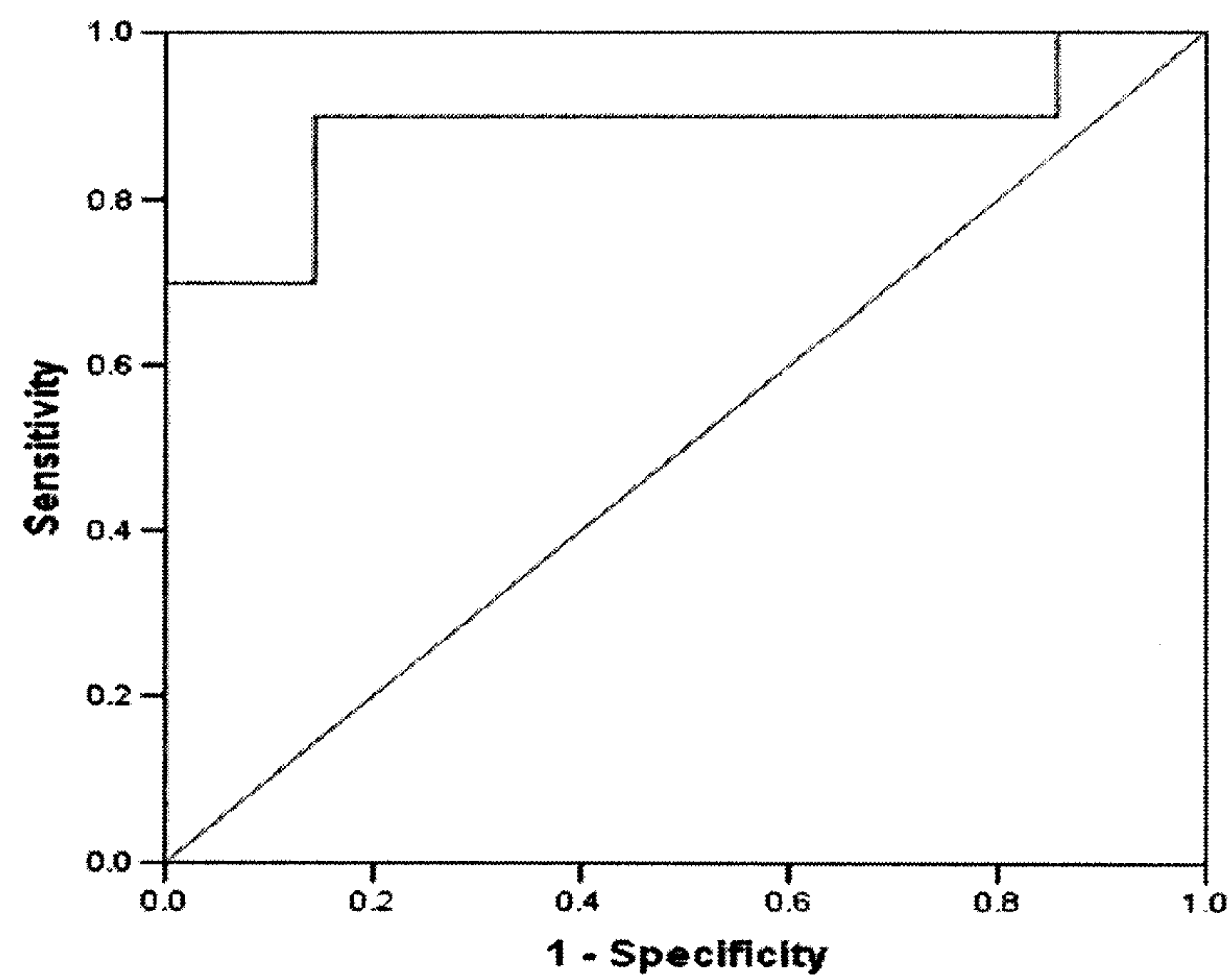


Figure 7c

24/133

Coordinates of the Curve

Test Result Variable(s): Probe 10

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-24.5800	1.000	1.000
-23.0973	1.000	.857
-22.4697	.900	.857
-20.4726	.900	.714
-18.5350	.900	.571
-18.1631	.900	.429
-15.2328	.900	.286
-10.6717	.900	.143
-8.4815	.800	.143
-7.7503	.700	.143
-7.2397	.700	.000
-7.0417	.600	.000
-6.8161	.500	.000
-6.4752	.400	.000
-6.1770	.300	.000
-5.0643	.200	.000
-3.3700	.100	.000
-1.7508	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

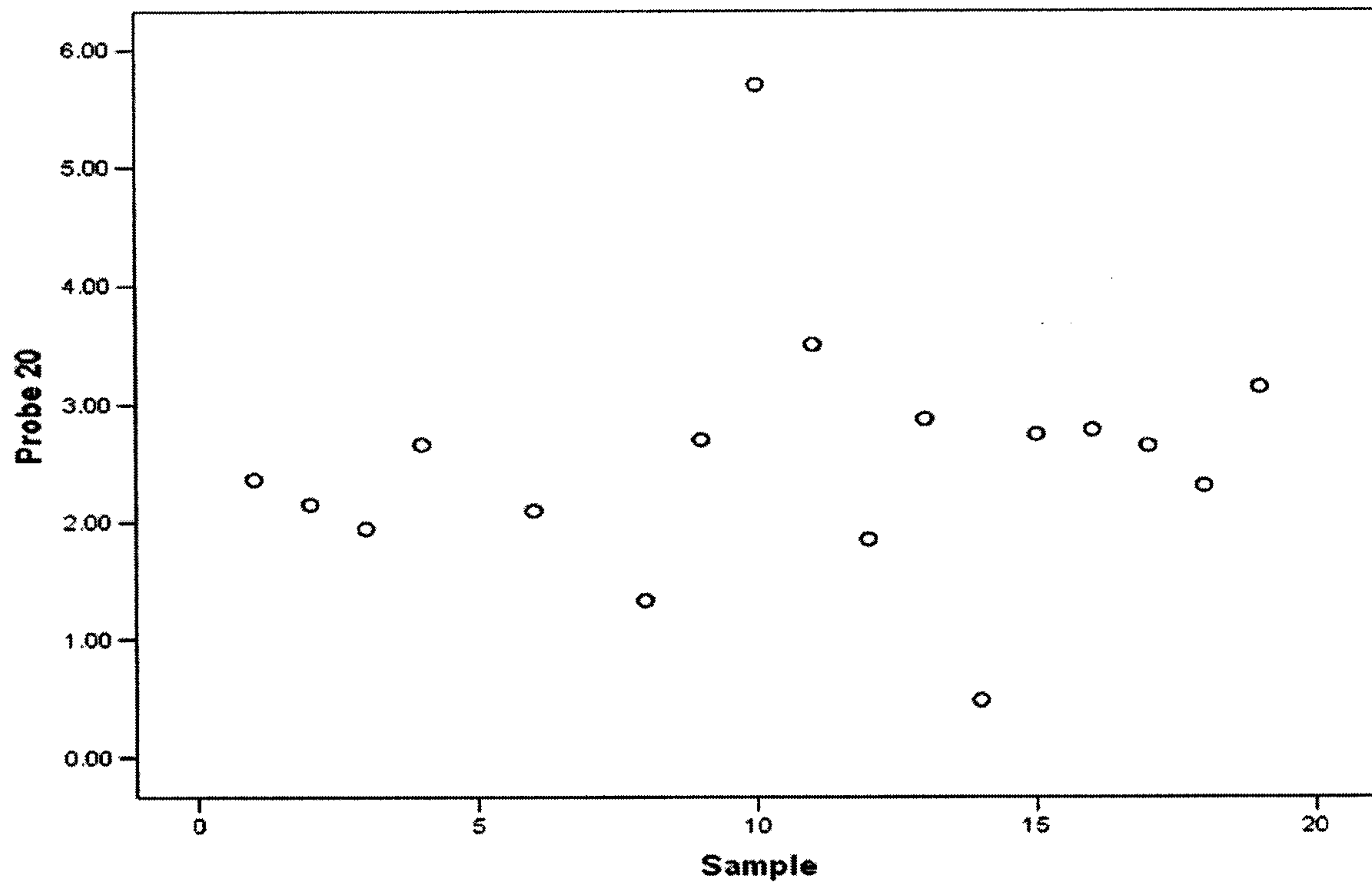
Test Result Variable(s): Probe 10

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.886	.089	.008	.712	1.060

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 7c (cond.)

25/133



ROC Curve

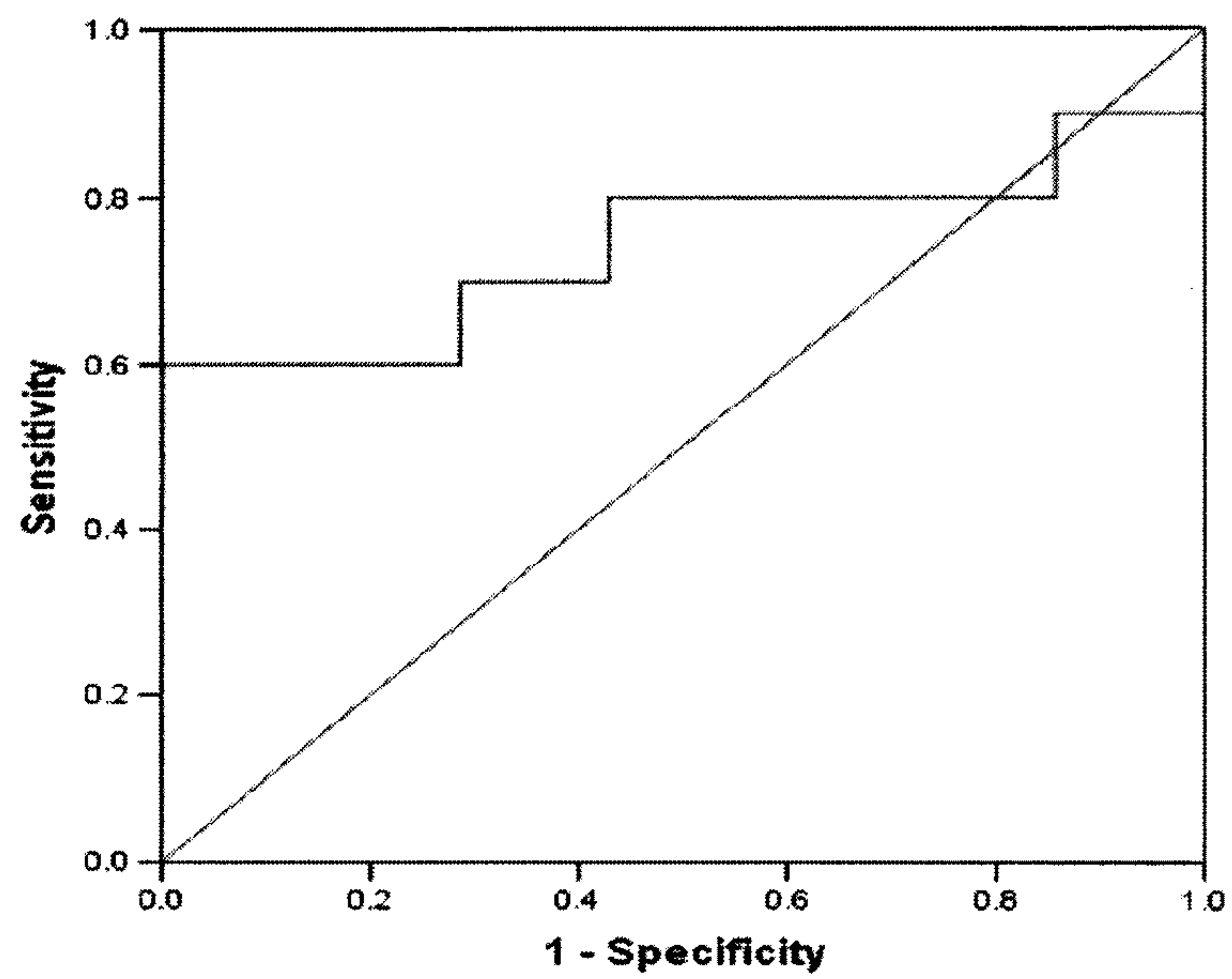


Figure 7d

26/133

Coordinates of the Curve

Test Result Variable(s): Probe 20

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-.5169	1.000	1.000
.9068	.900	1.000
1.5912	.900	.857
1.8981	.800	.857
2.0188	.800	.714
2.1229	.800	.571
2.2297	.800	.429
2.3347	.700	.429
2.5071	.700	.286
2.6562	.600	.286
2.6809	.600	.143
2.7242	.600	.000
2.7672	.500	.000
2.8328	.400	.000
3.0151	.300	.000
3.3308	.200	.000
4.6060	.100	.000
6.7022	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

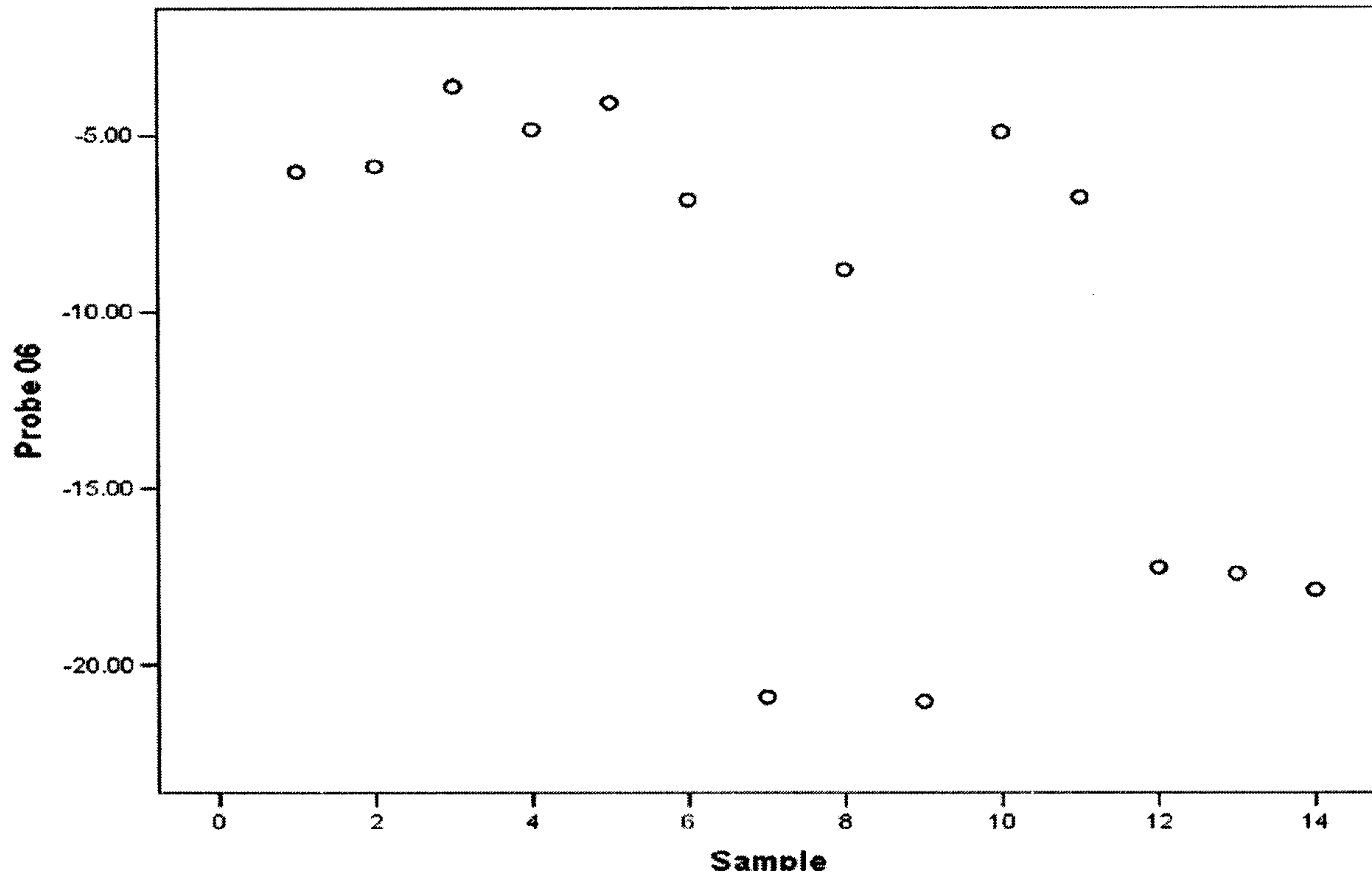
Test Result Variable(s): Probe 20

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.743	.125	.097	.498	.988

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 7d (cond.)

27/133



ROC Curve

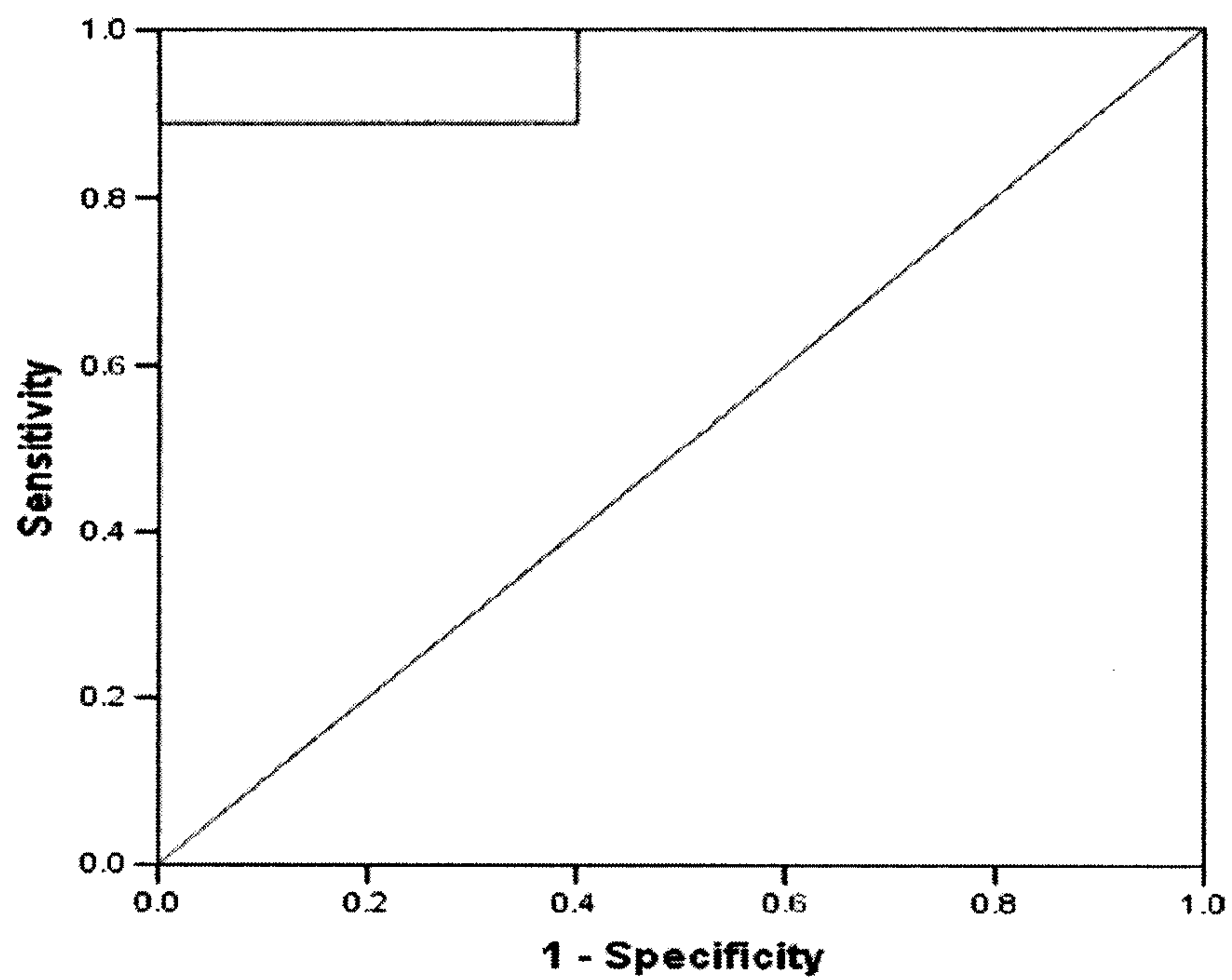


Figure 8a

28/133

Coordinates of the Curve

Test Result Variable(s): Probe 06

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-22.0392	.000	.000
-20.9814	.111	.000
-19.4099	.222	.000
-17.6643	.333	.000
-17.3445	.444	.000
-13.0322	.556	.000
-7.8187	.667	.000
-6.7994	.778	.000
-6.3994	.889	.000
-5.9531	.889	.200
-5.3975	.889	.400
-4.8747	1.000	.400
-4.4546	1.000	.600
-3.8466	1.000	.800
-2.6149	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Probe 06

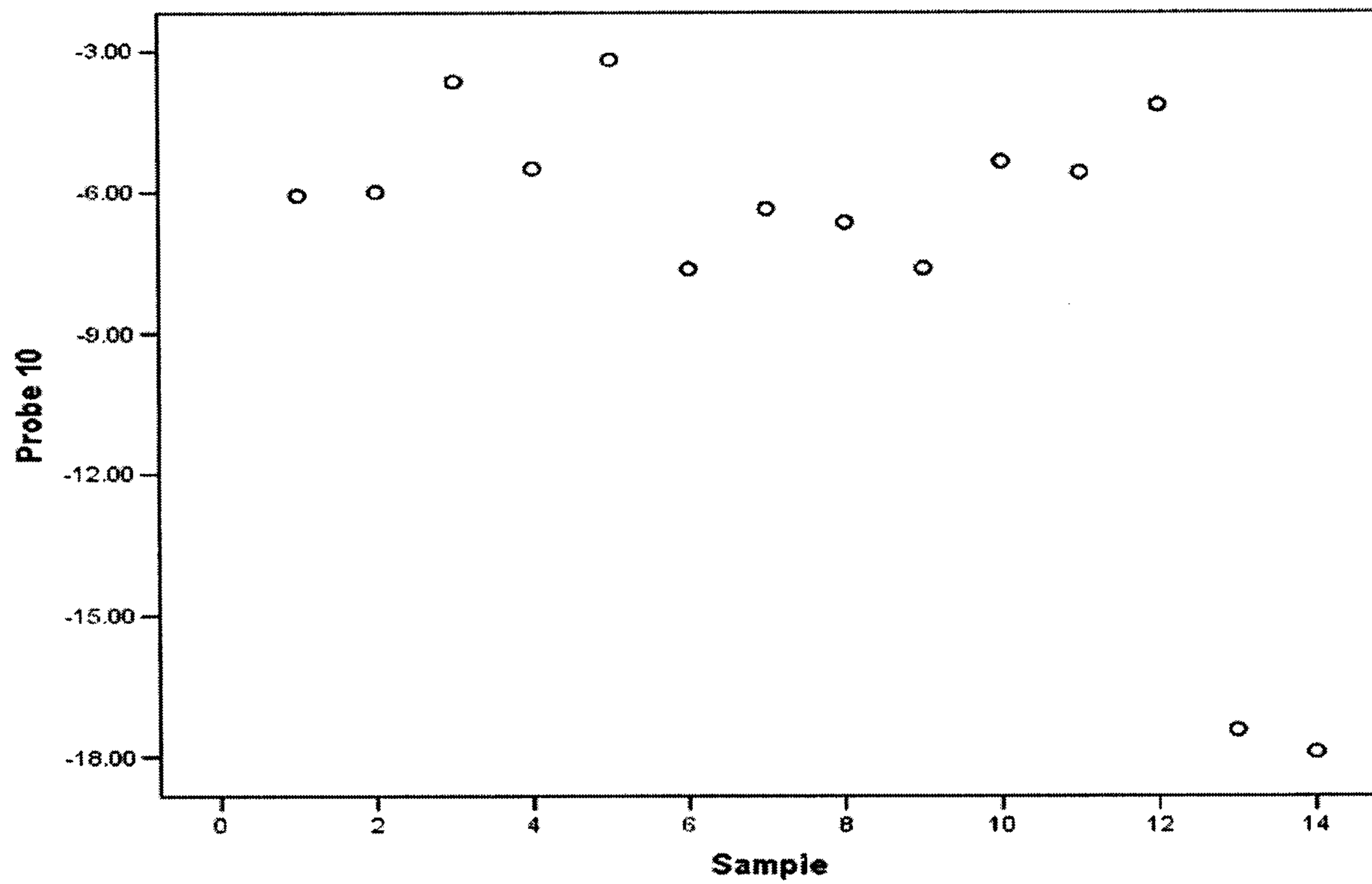
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.956	.054	.006	.850	1.061

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 8a (cond.)

29/133



ROC Curve

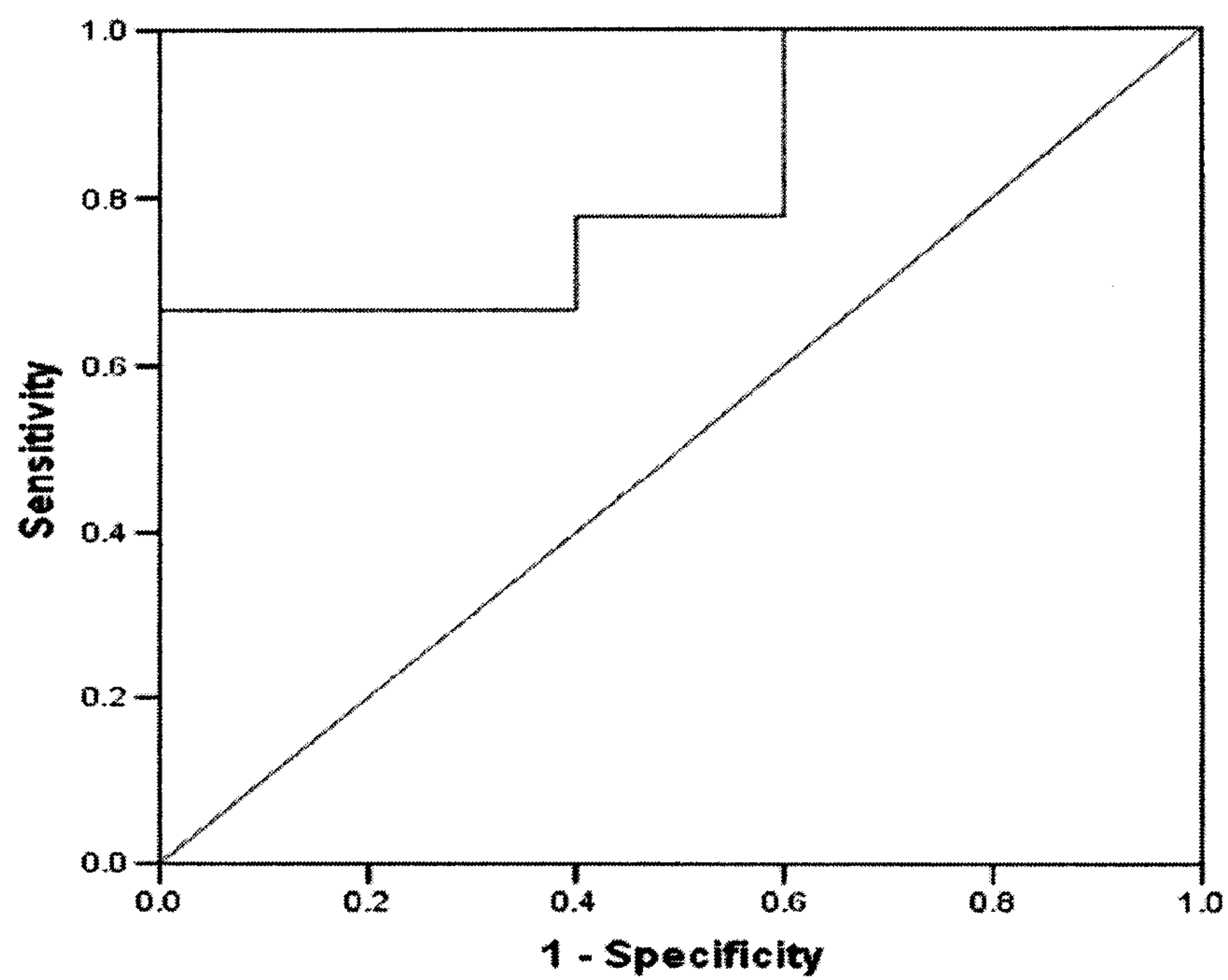


Figure 8b

30/133

Coordinates of the Curve

Test Result Variable(s): Probe 10

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-18.8961	.000	.000
-17.6643	.111	.000
-12.5362	.222	.000
-7.6318	.333	.000
-7.1352	.444	.000
-6.5045	.556	.000
-6.2157	.667	.000
-6.0322	.667	.200
-5.7932	.667	.400
-5.5472	.778	.400
-5.4308	.778	.600
-4.7572	.889	.600
-3.9035	1.000	.600
-3.4191	1.000	.800
-2.1871	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

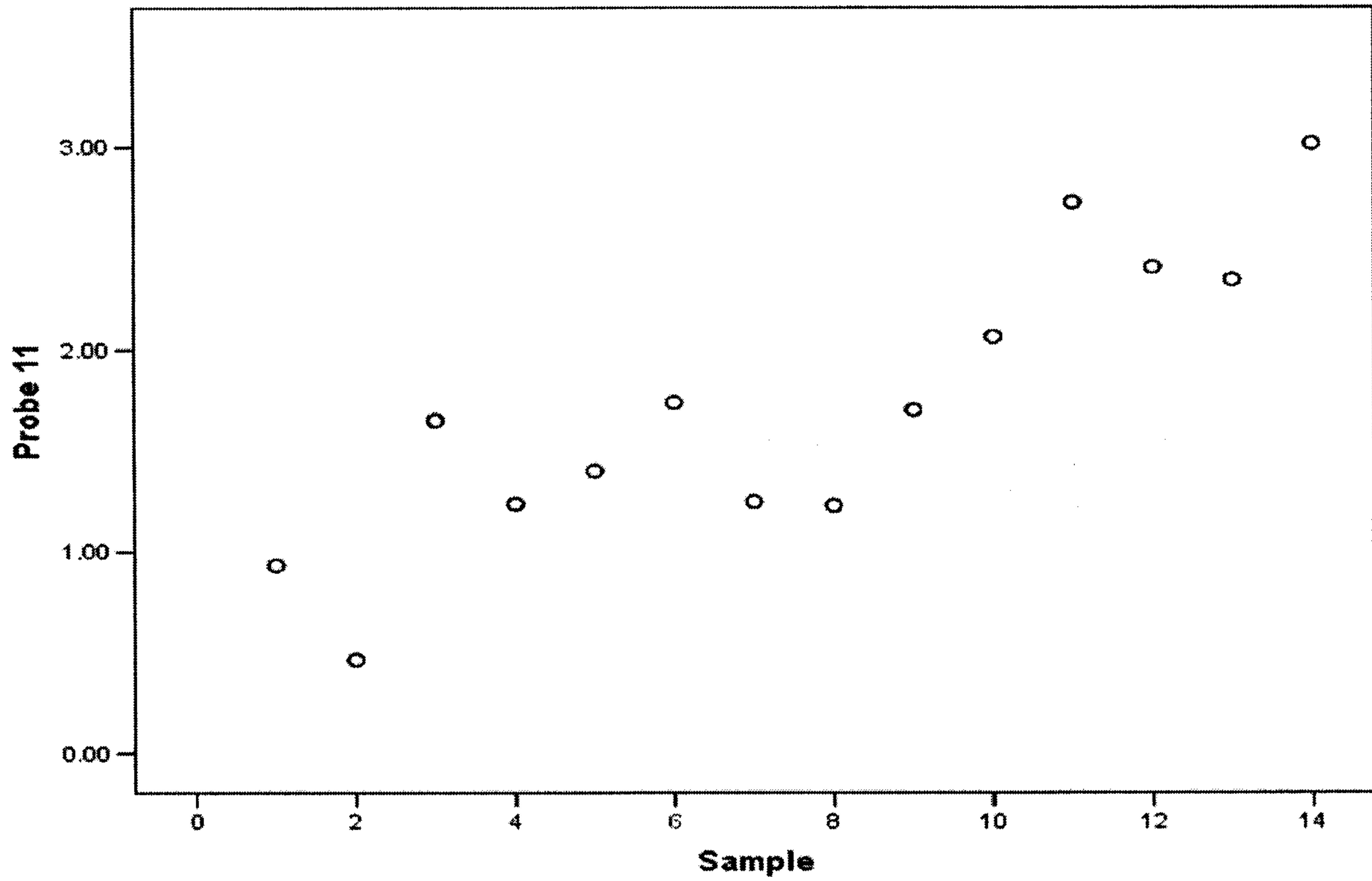
Test Result Variable(s): Probe 10

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.822	.115	.053	.597	1.047

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 8b (cond.)

31/133



ROC Curve

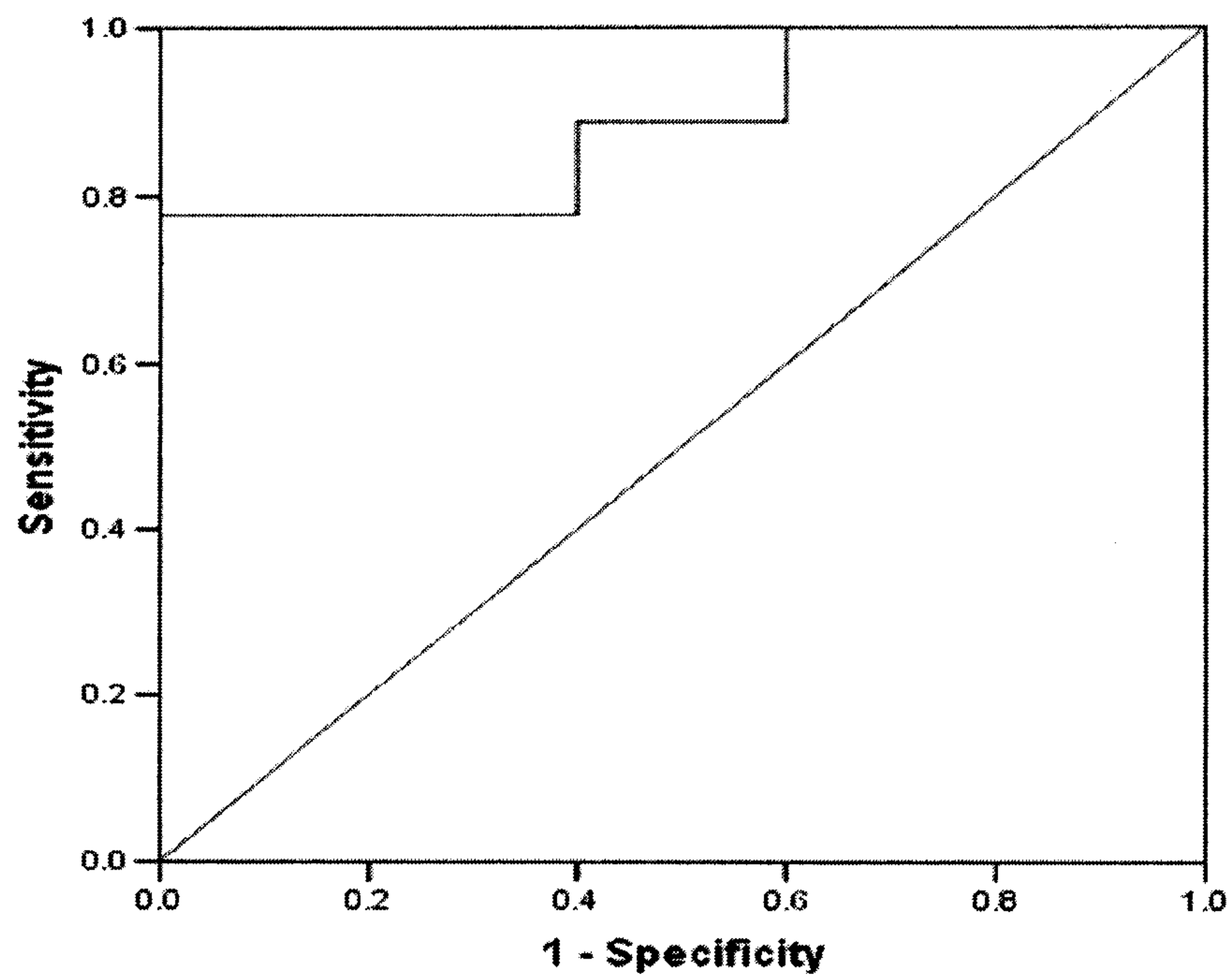


Figure 8c

32/133

Coordinates of the Curve

Test Result Variable(s): Probe 11

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-.5371	1.000	1.000
.6980	1.000	.800
1.0810	1.000	.600
1.2329	.889	.600
1.2424	.889	.400
1.3246	.778	.400
1.5257	.778	.200
1.6762	.778	.000
1.7214	.667	.000
1.9017	.556	.000
2.2043	.444	.000
2.3772	.333	.000
2.5673	.222	.000
2.8707	.111	.000
4.0156	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

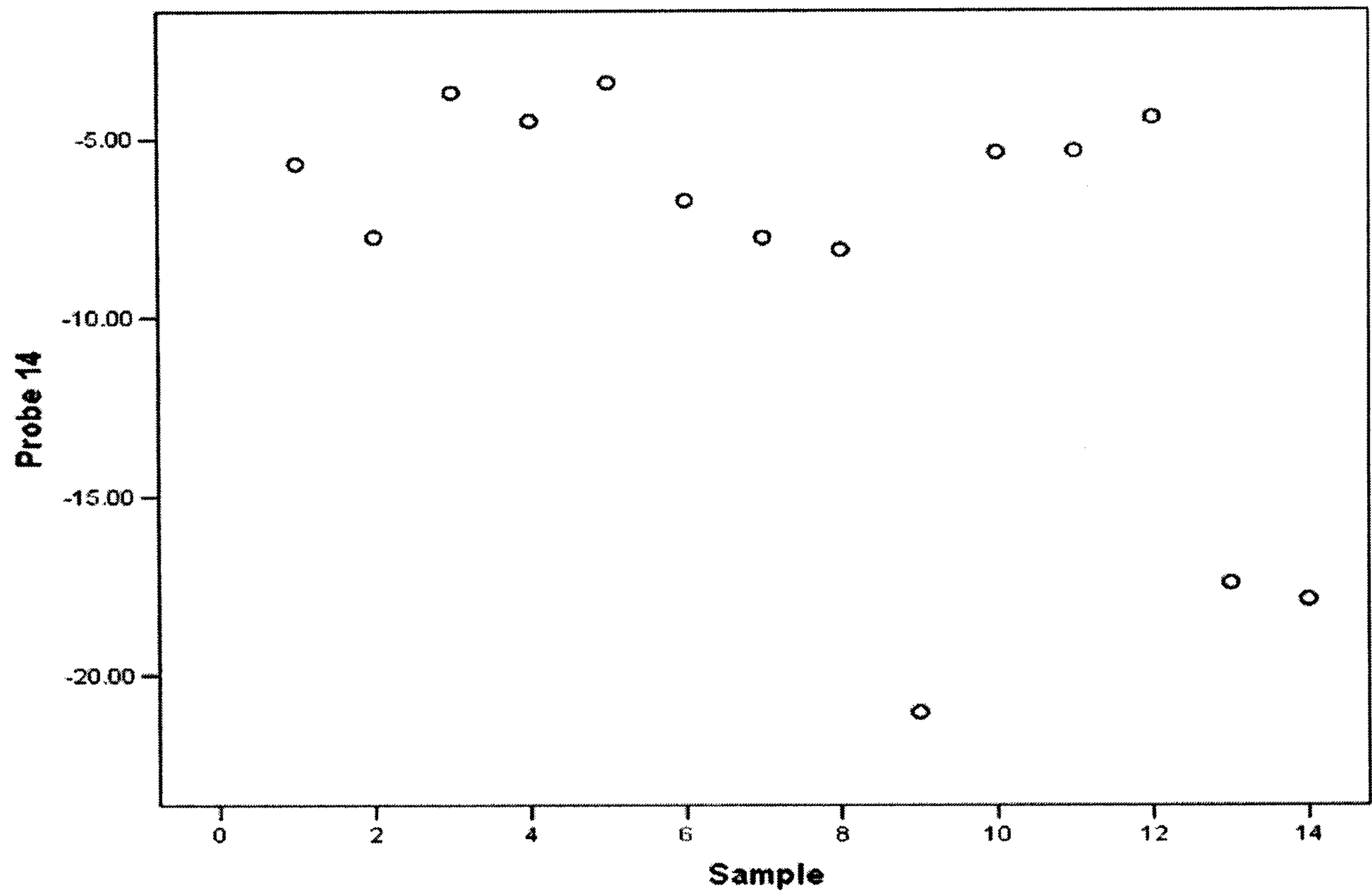
Test Result Variable(s): Probe 11

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.889	.089	.020	.714	1.064

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 8c (cond.)

33/133



ROC Curve

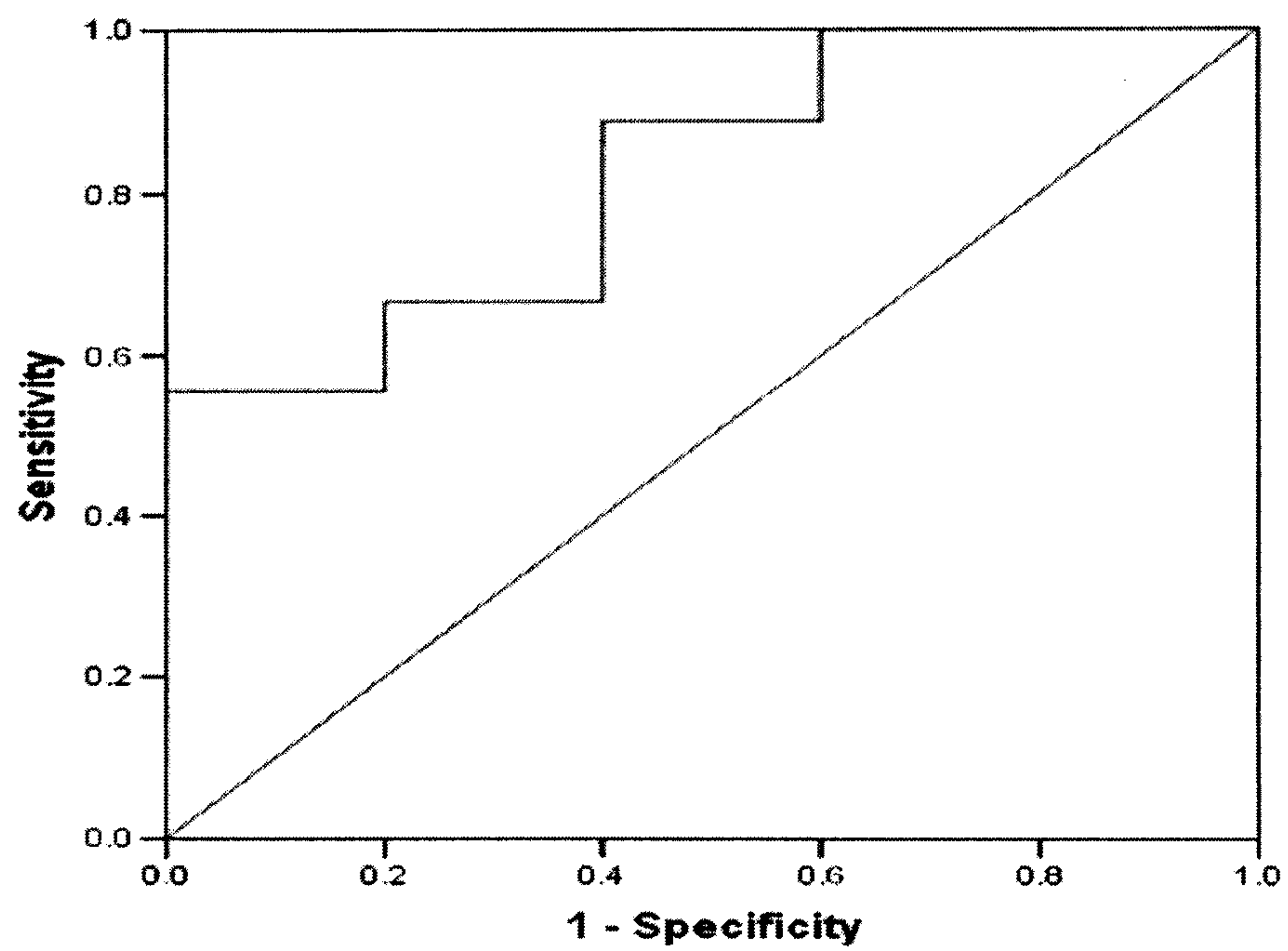


Figure 8d

34/133

Coordinates of the Curve

Test Result Variable(s): Probe 14

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-22.0392	.000	.000
-19.4676	.111	.000
-17.6643	.222	.000
-12.7717	.333	.000
-7.9424	.444	.000
-7.7602	.556	.000
-7.2406	.556	.200
-6.2127	.667	.200
-5.5389	.667	.400
-5.3642	.778	.400
-4.9118	.889	.400
-4.4384	.889	.600
-4.0421	1.000	.600
-3.5533	1.000	.800
-2.4171	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

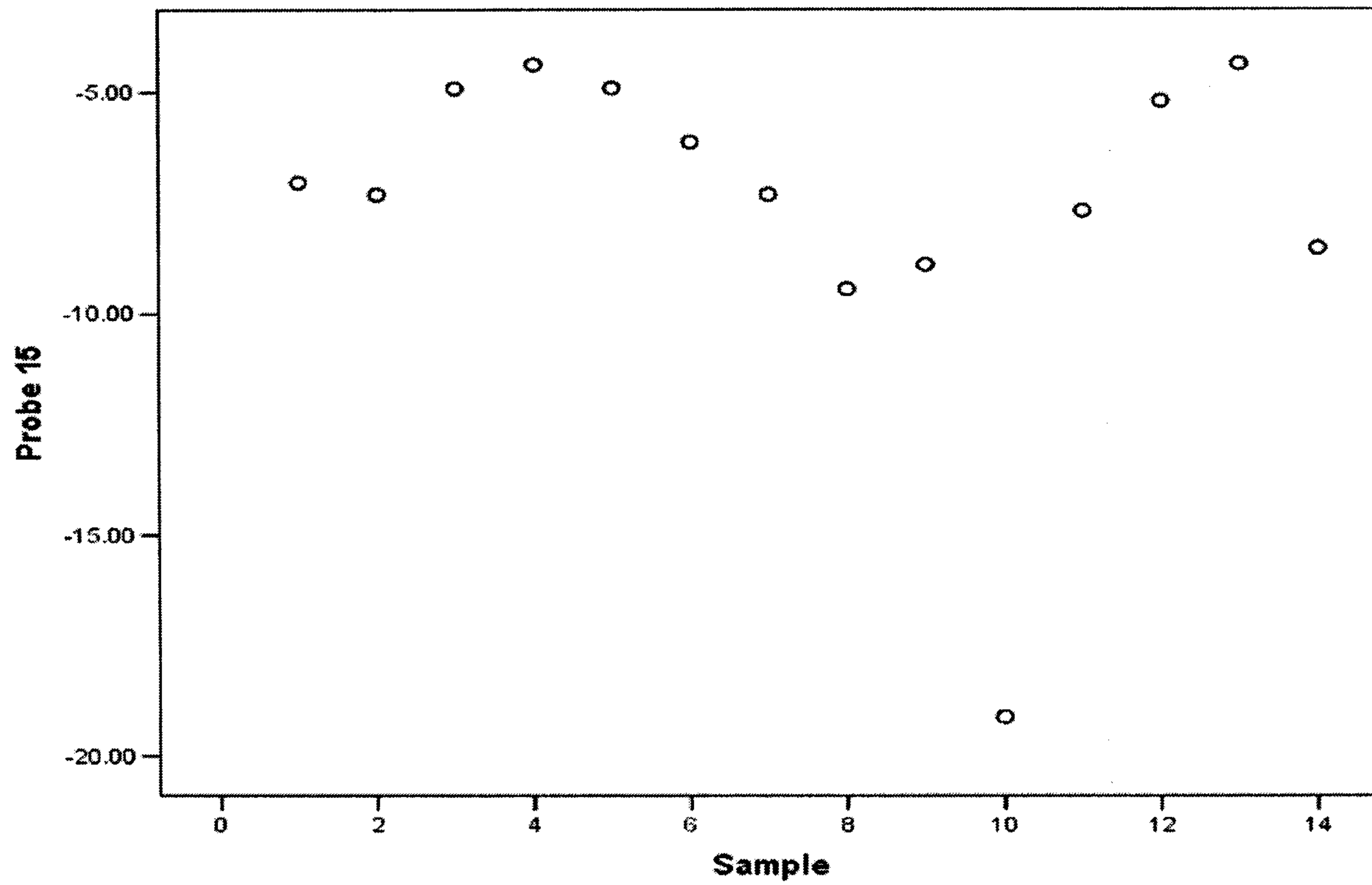
Test Result Variable(s): Probe 14

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.822	.116	.053	.596	1.049

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 8d (cond.)

35/133



ROC Curve

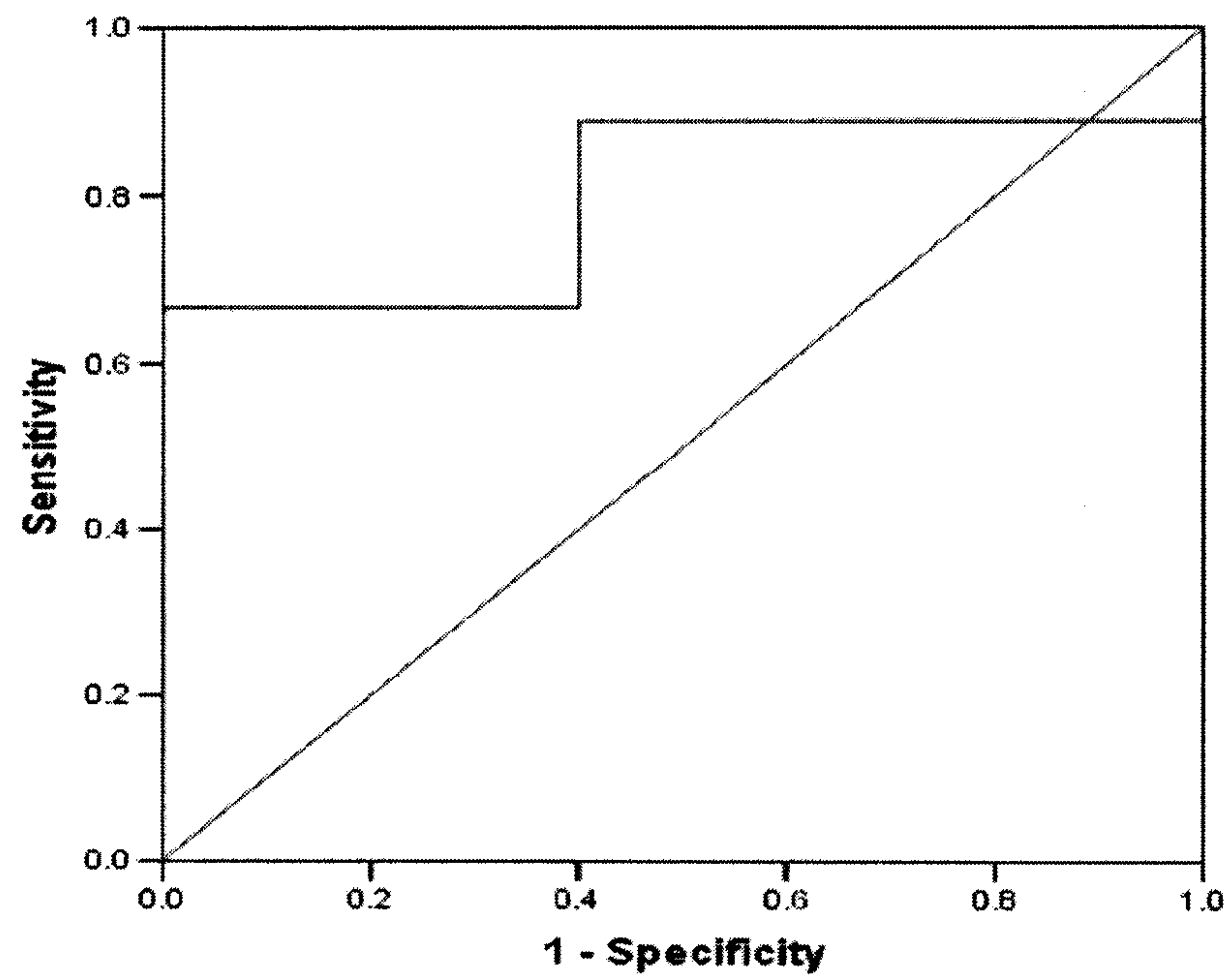


Figure 8e

36/133

Coordinates of the Curve

Test Result Variable(s): Probe 15

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-20.1142	.000	.000
-14.2809	.111	.000
-9.1717	.222	.000
-8.7108	.333	.000
-8.1027	.444	.000
-7.4967	.556	.000
-7.3107	.667	.000
-7.1709	.667	.200
-6.5789	.667	.400
-5.6633	.778	.400
-5.0574	.889	.400
-4.9067	.889	.600
-4.6344	.889	.800
-4.3629	.889	1.000
-3.3582	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

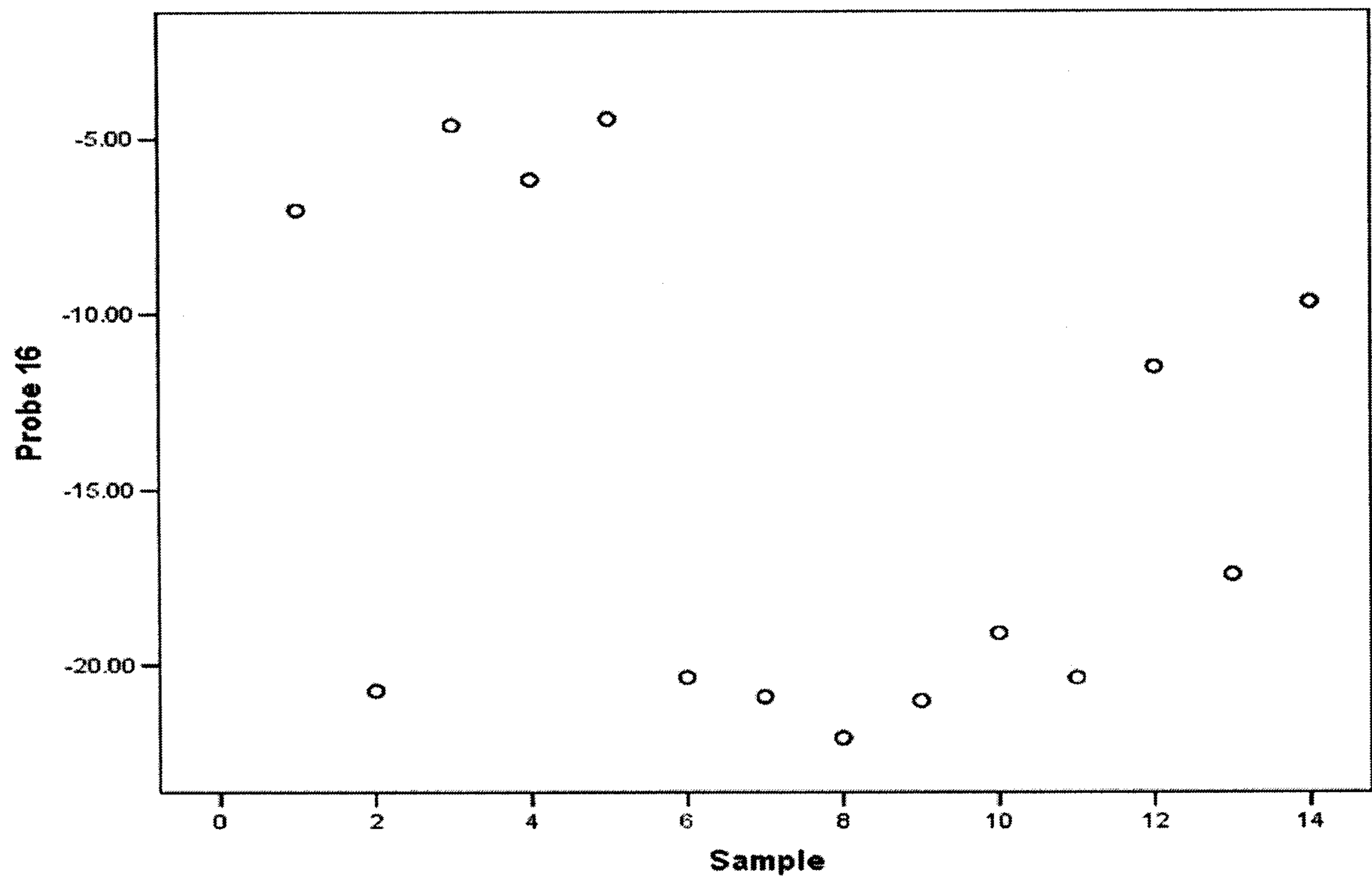
Test Result Variable(s): Probe 15

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.800	.123	.072	.559	1.041

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 8e (cond.)

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ROC Curve

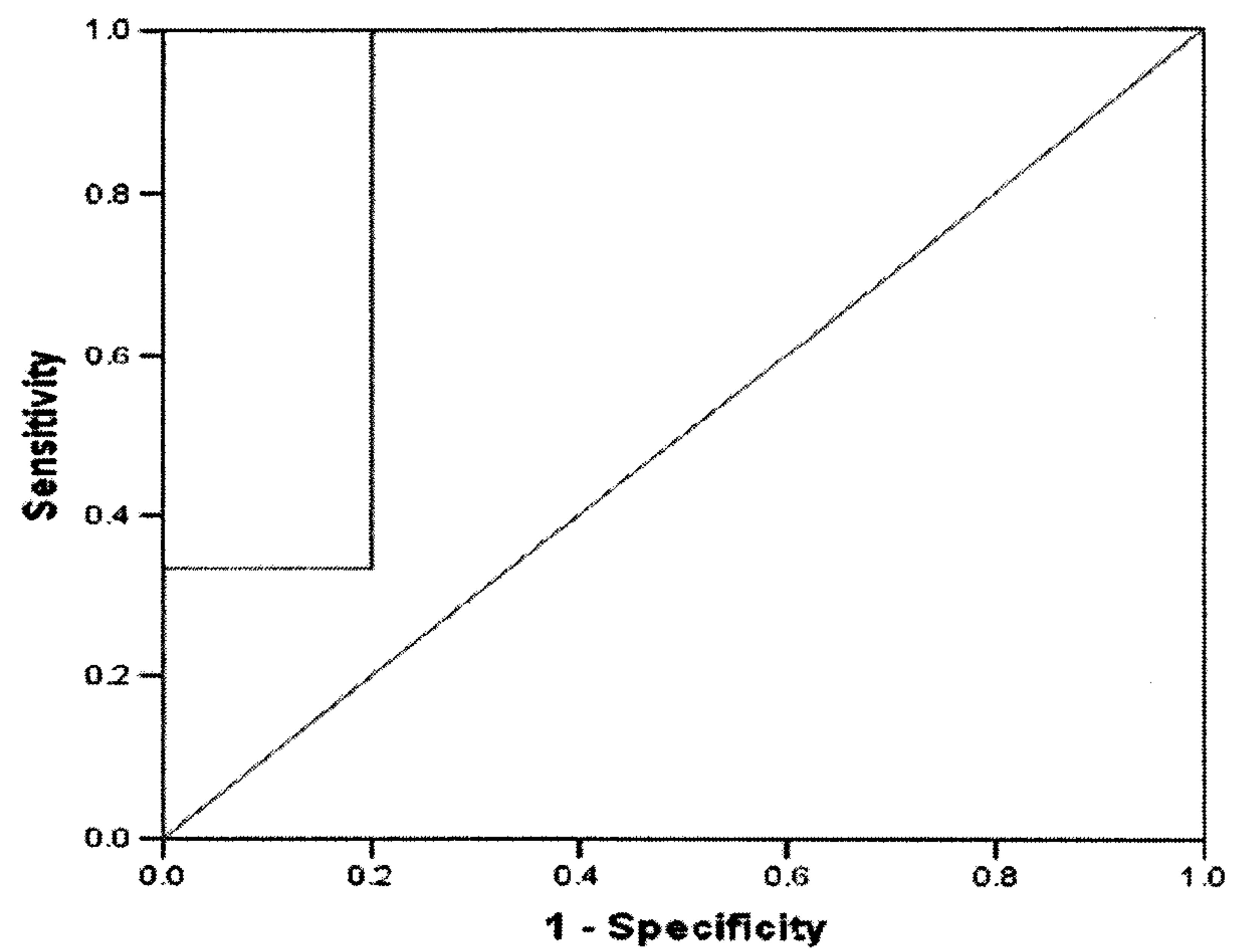


Figure 8f

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Coordinates of the Curve

Test Result Variable(s): Probe 16

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-23.0959	.000	.000
-21.5675	.111	.000
-20.9814	.222	.000
-20.8346	.333	.000
-20.5630	.333	.200
-20.3762	.444	.200
-19.7431	.556	.200
-18.2733	.667	.200
-14.4760	.778	.200
-10.5963	.889	.200
-8.3543	1.000	.200
-6.6018	1.000	.400
-5.3915	1.000	.600
-4.5308	1.000	.800
-3.4469	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

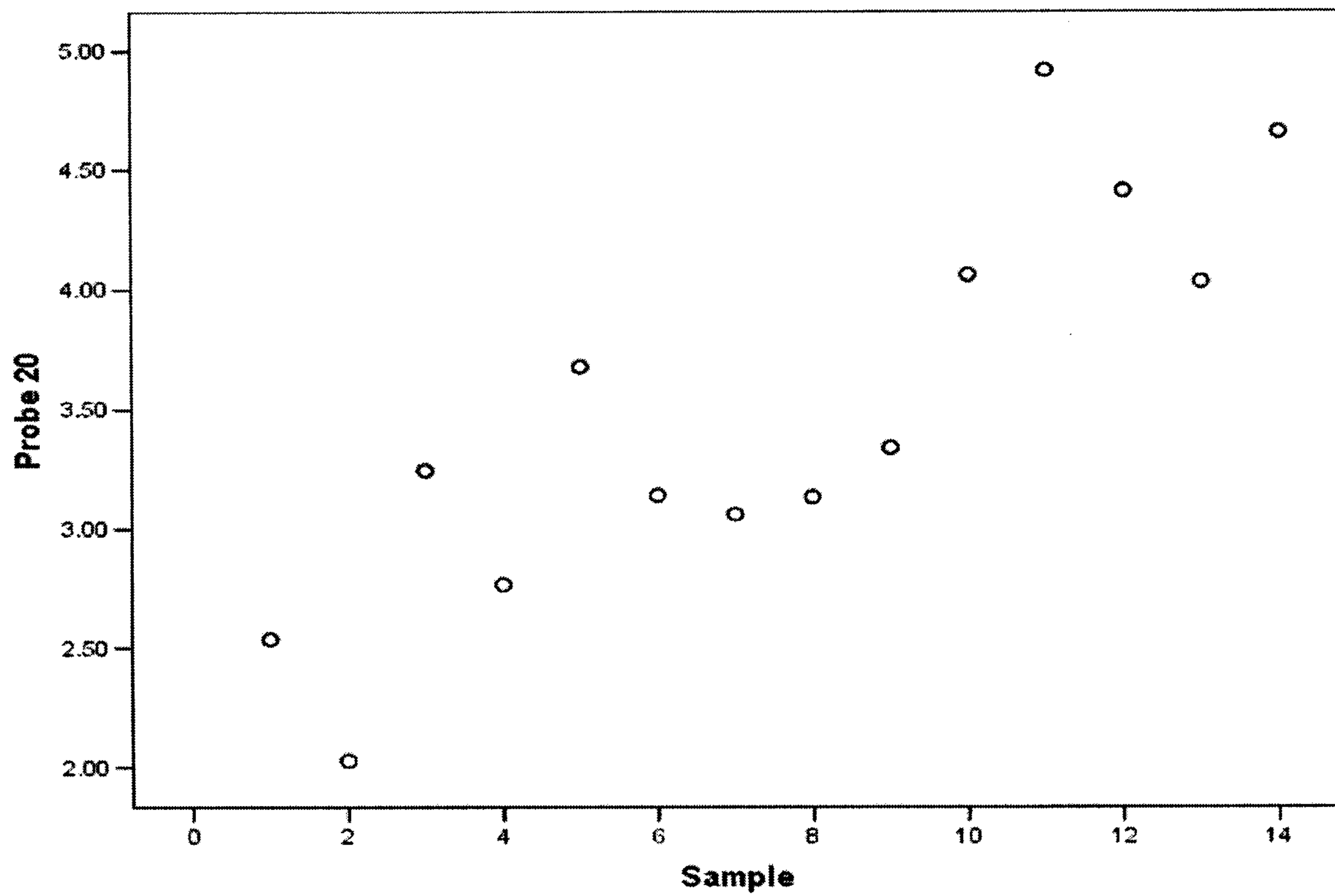
Test Result Variable(s): Probe 16

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.867	.126	.028	.619	1.115

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 8f (cond.)

39/133



ROC Curve

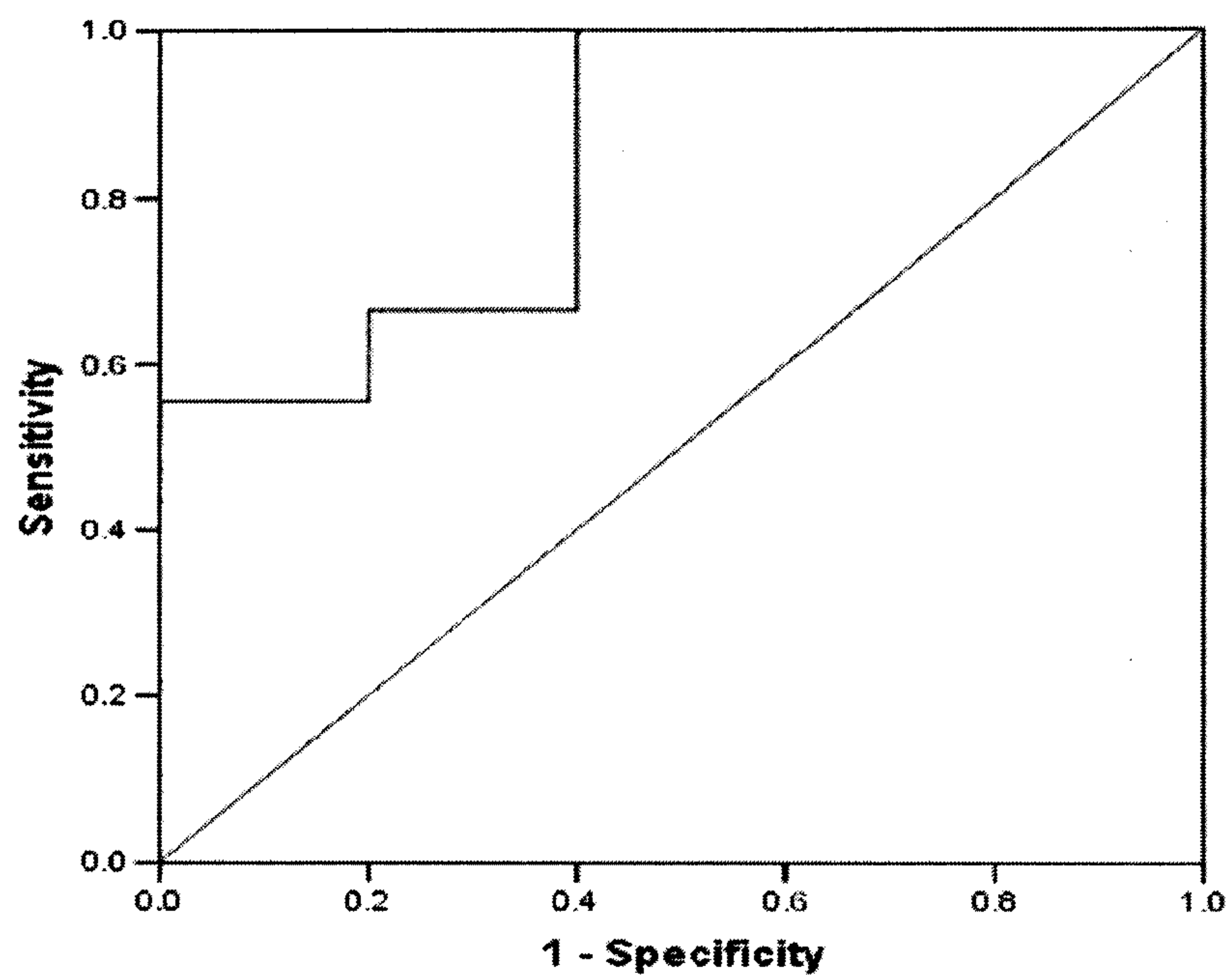


Figure 8g

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Coordinates of the Curve

Test Result Variable(s): Probe 20

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
1.0284	1.000	1.000
2.2833	1.000	.800
2.6520	1.000	.600
2.9137	1.000	.400
3.0975	.889	.400
3.1367	.778	.400
3.1915	.667	.400
3.2910	.667	.200
3.5084	.556	.200
3.8538	.556	.000
4.0448	.444	.000
4.2349	.333	.000
4.5324	.222	.000
4.7853	.111	.000
5.9158	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Probe 20

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.844	.112	.039	.625	1.064

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 8g (cond.)

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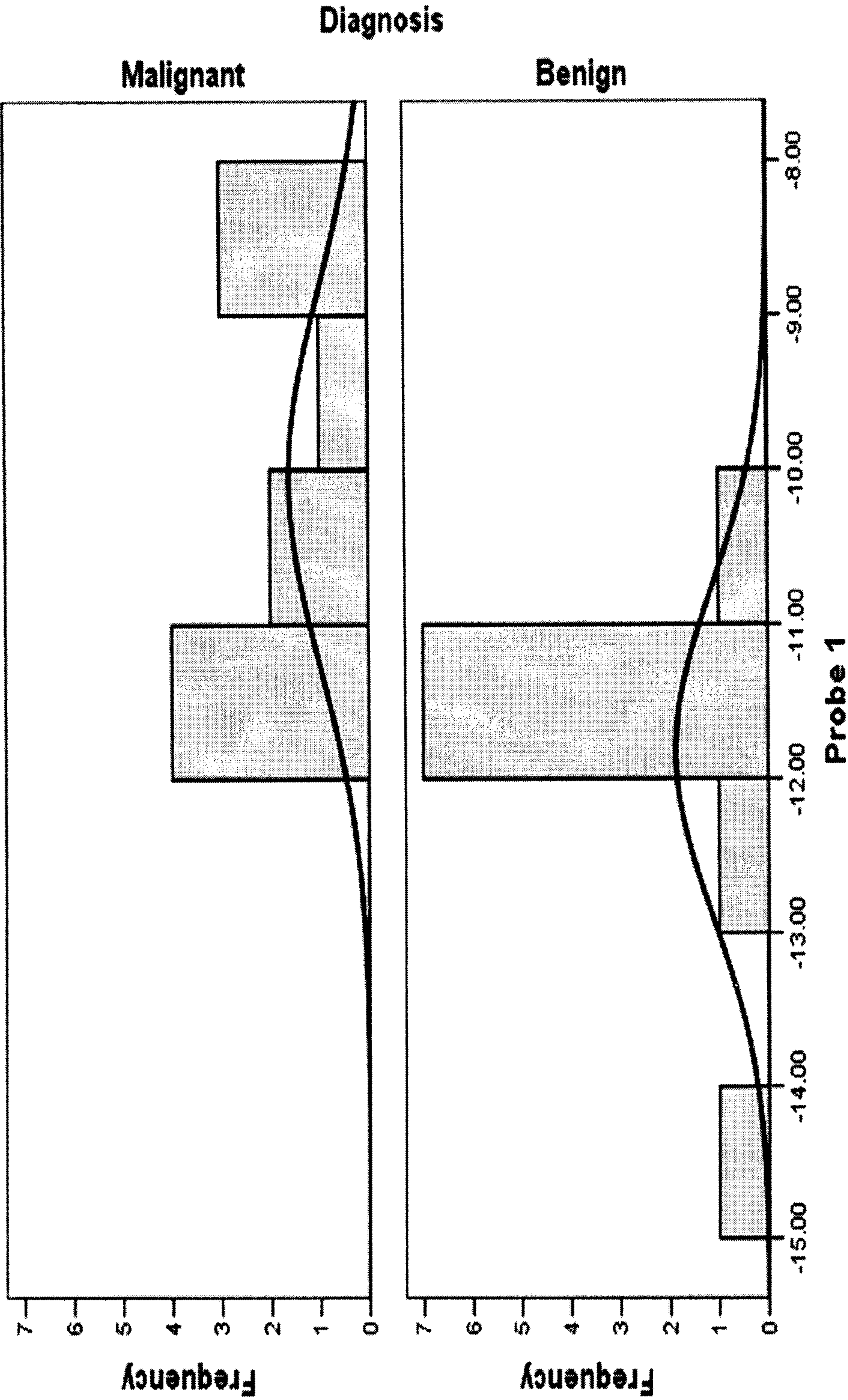


Figure 9a

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Coordinates of the Curve

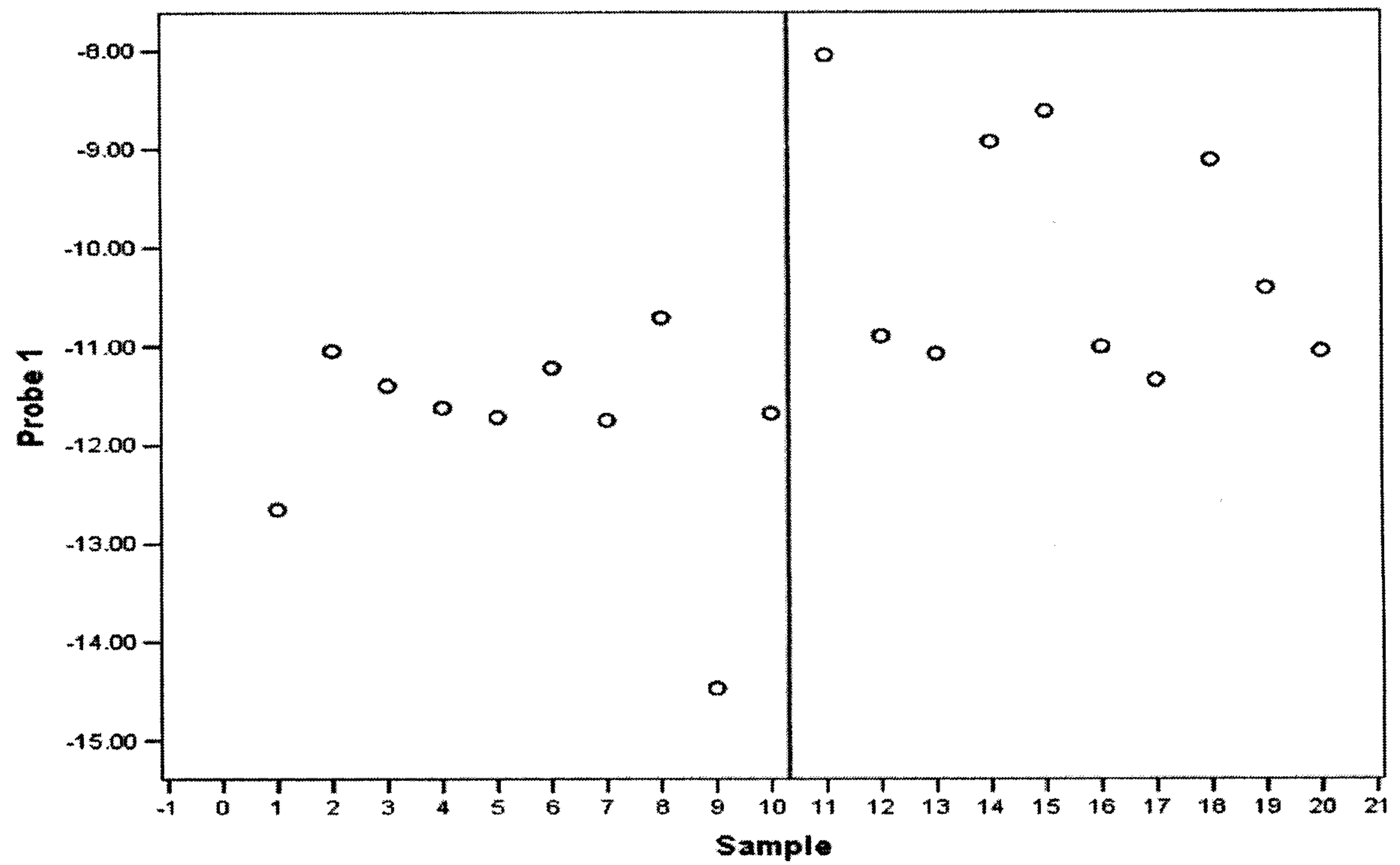
Test Result Variable(s): Probe 1

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-15.4761	1.000	1.000
-13.5649	1.000	.900
-12.2015	1.000	.800
-11.7327	1.000	.700
-11.6981	1.000	.600
-11.6511	1.000	.500
-11.5103	1.000	.400
-11.3723	1.000	.300
-11.2832	.900	.300
-11.1503	.900	.200
-11.0681	.800	.200
-11.0514	.700	.200
-11.0285	.700	.100
-10.9575	.600	.100
-10.8113	.500	.100
-10.5685	.500	.000
-9.7680	.400	.000
-9.0247	.300	.000
-8.7768	.200	.000
-8.3329	.100	.000
-7.0448	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 9a (cond.)

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ROC Curve

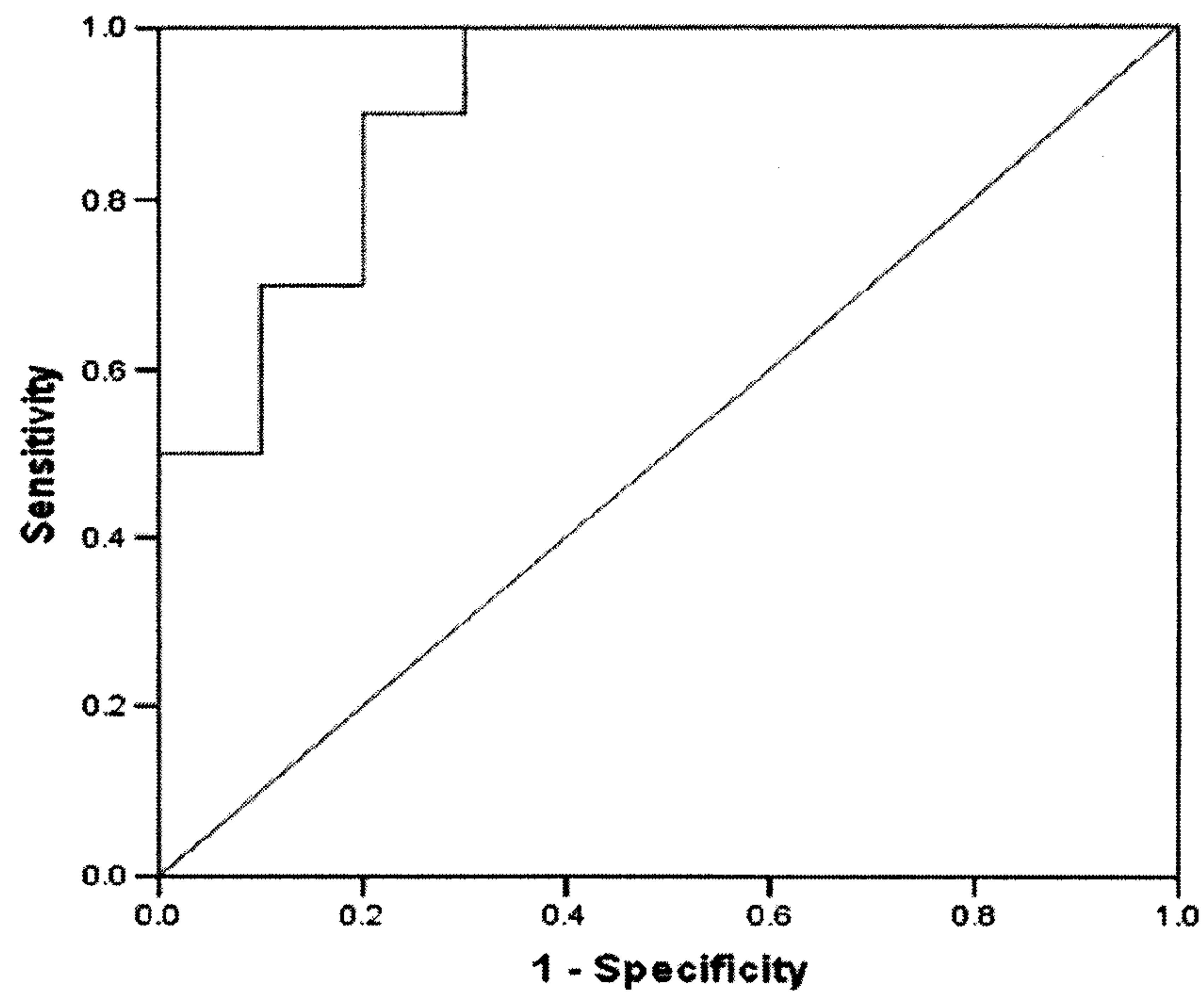


Figure 9a (cond.)

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Area Under the Curve

Test Result Variable(s): Probe 1

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.910	.065	.002	.783	1.037

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 9a (cond.)

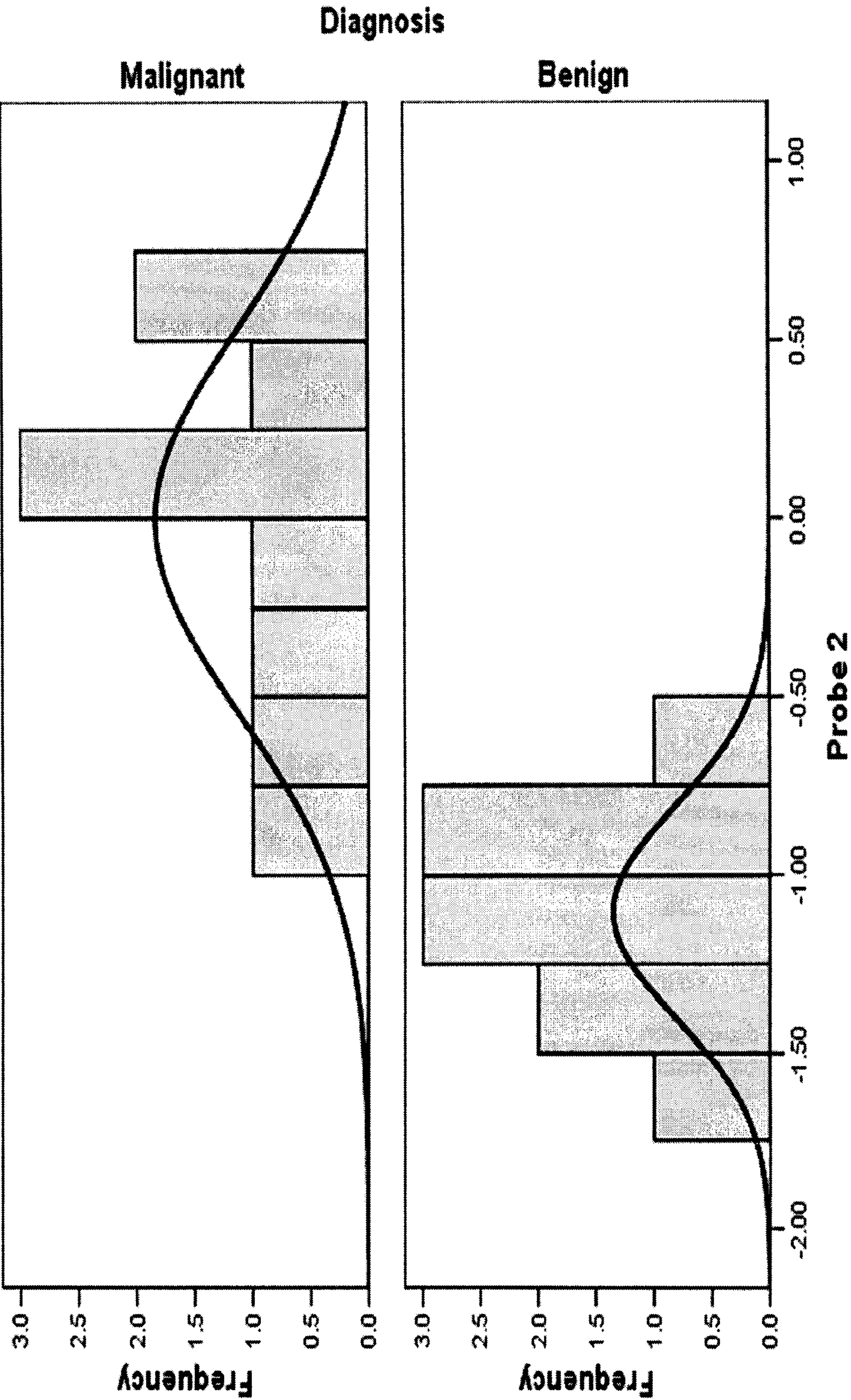


Figure 9b

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Coordinates of the Curve

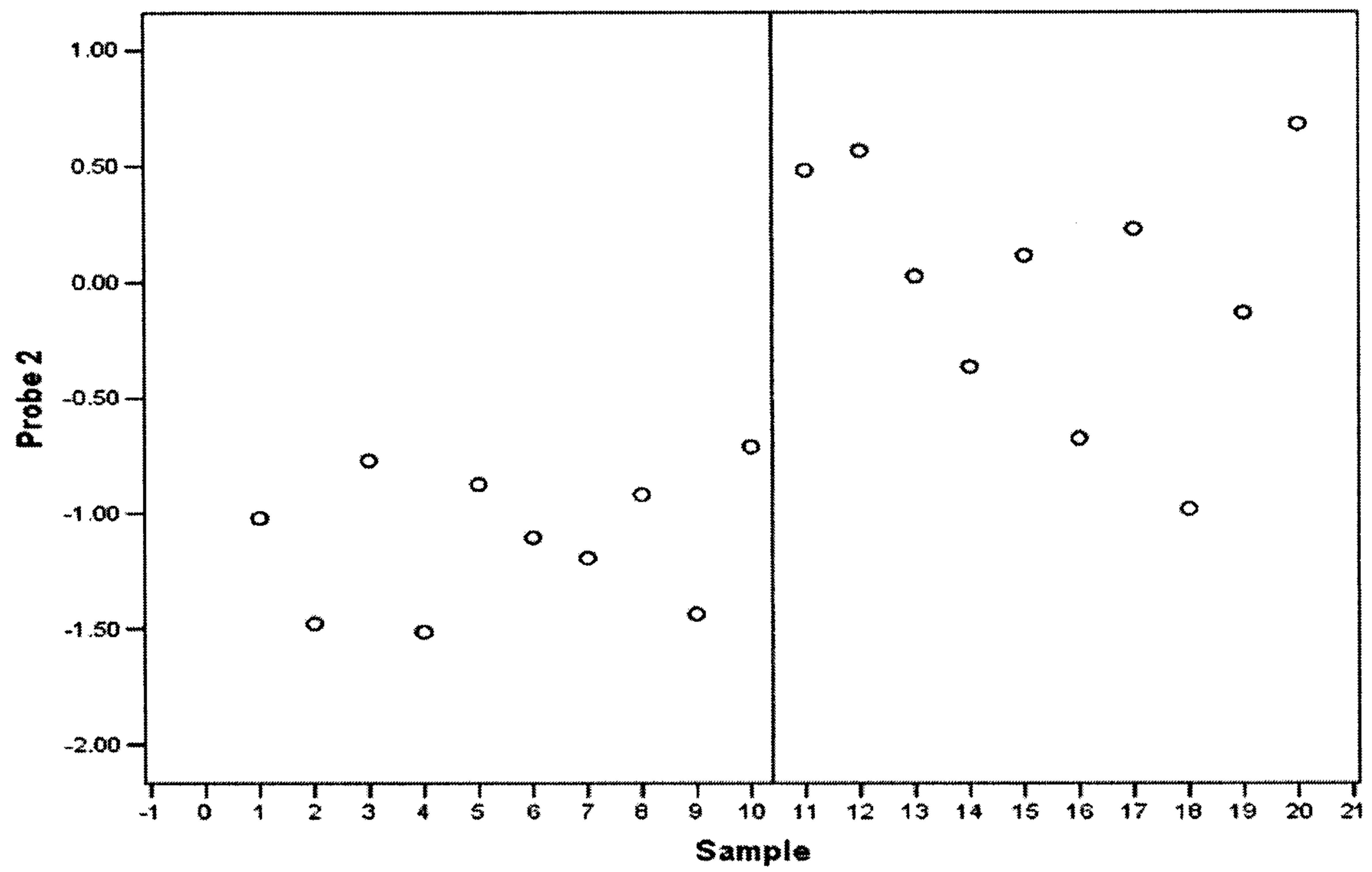
Test Result Variable(s): Probe 2

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-2.5142	1.000	1.000
-1.4962	1.000	.900
-1.4577	1.000	.800
-1.3167	1.000	.700
-1.1509	1.000	.600
-1.0633	1.000	.500
-1.0013	1.000	.400
-.9511	.900	.400
-.8981	.900	.300
-.8244	.900	.200
-.7437	.900	.100
-.6962	.900	.000
-.5223	.800	.000
-.2498	.700	.000
-.0539	.600	.000
.0701	.500	.000
.1717	.400	.000
.3552	.300	.000
.5236	.200	.000
.6224	.100	.000
1.6797	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 9b (cond.)

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ROC Curve

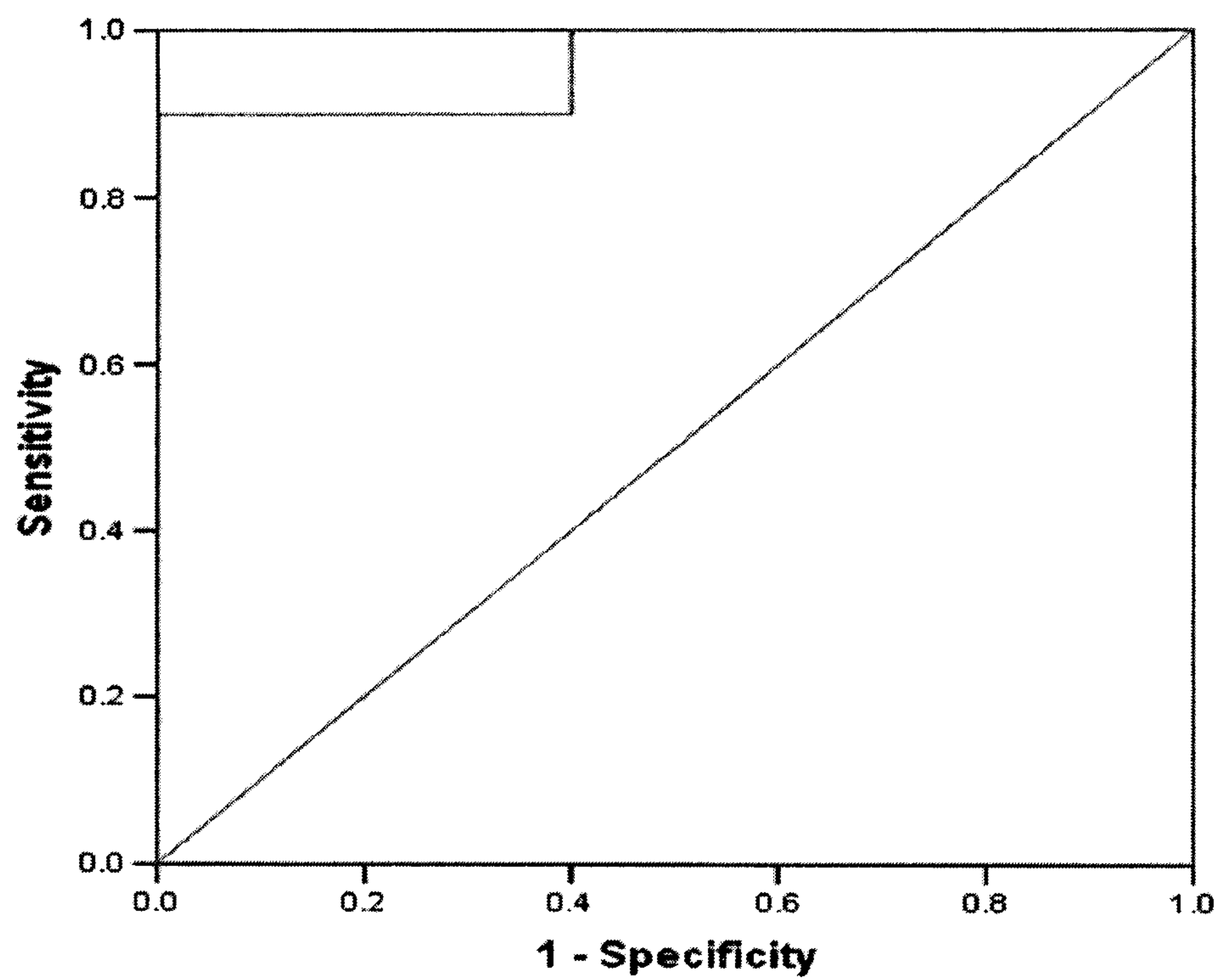


Figure 9b (cond.)

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Area Under the Curve

Test Result Variable(s): Probe 2

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.960	.044	.001	.875	1.045

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 9b (cond.)

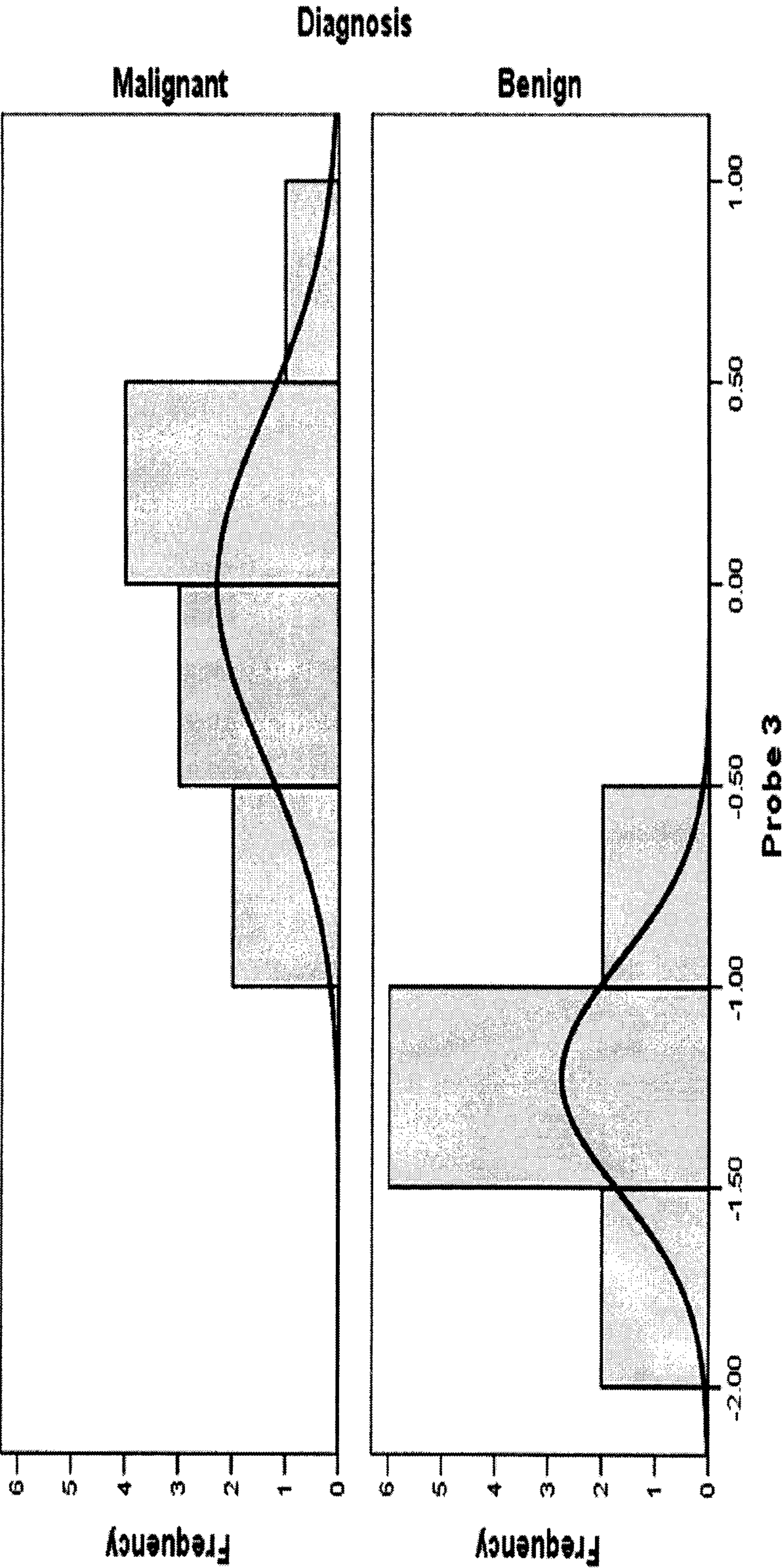


Figure 9c

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Coordinates of the Curve

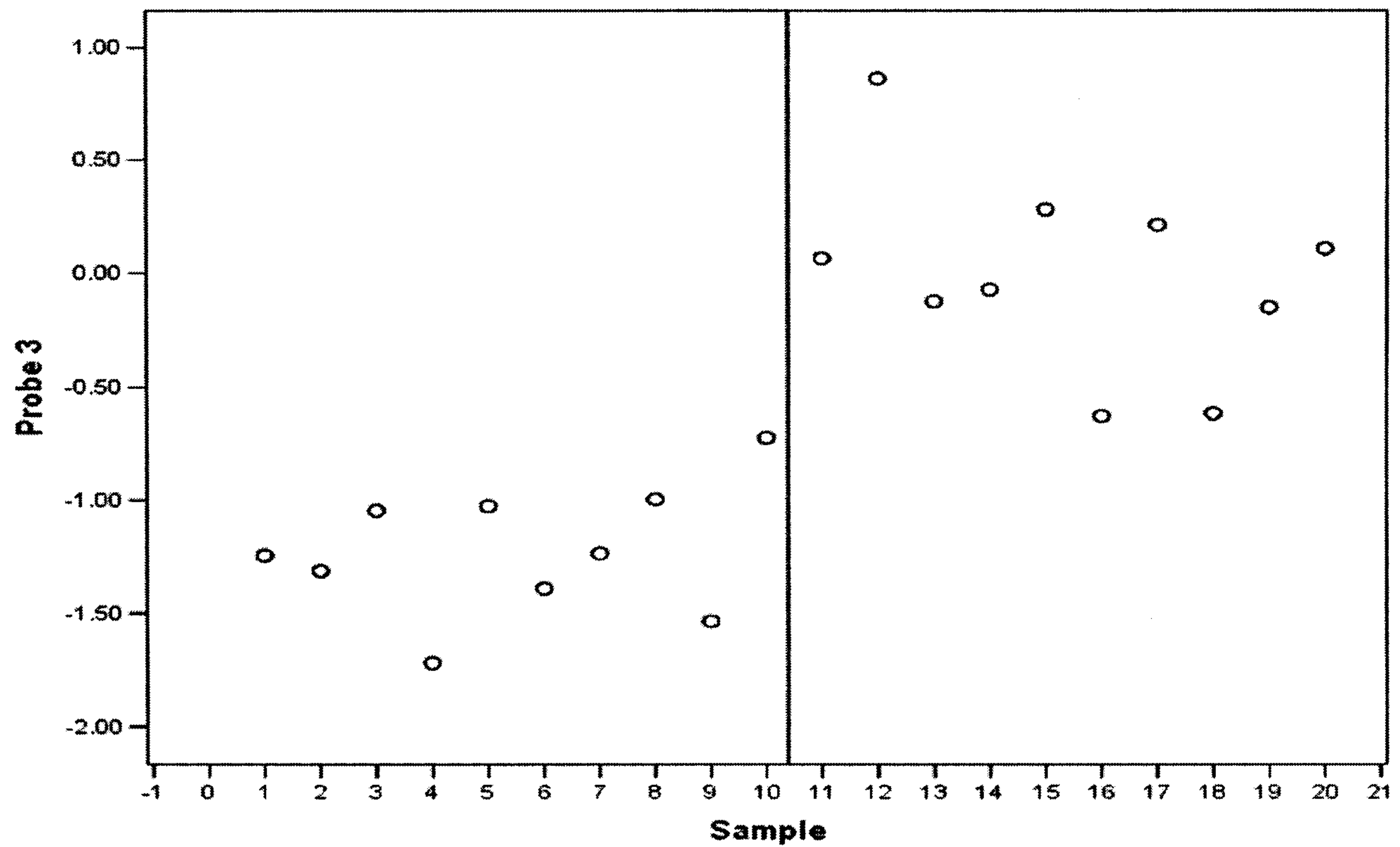
Test Result Variable(s): Probe 3

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-2.7188	1.000	1.000
-1.6268	1.000	.900
-1.4628	1.000	.800
-1.3513	1.000	.700
-1.2780	1.000	.600
-1.2393	1.000	.500
-1.1402	1.000	.400
-1.0353	1.000	.300
-1.0098	1.000	.200
-.8590	1.000	.100
-.6754	1.000	.000
-.6215	.900	.000
-.3833	.800	.000
-.1380	.700	.000
-.0985	.600	.000
-.0043	.500	.000
.0854	.400	.000
.1595	.300	.000
.2471	.200	.000
.5721	.100	.000
1.8621	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 9c (cond.)

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ROC Curve

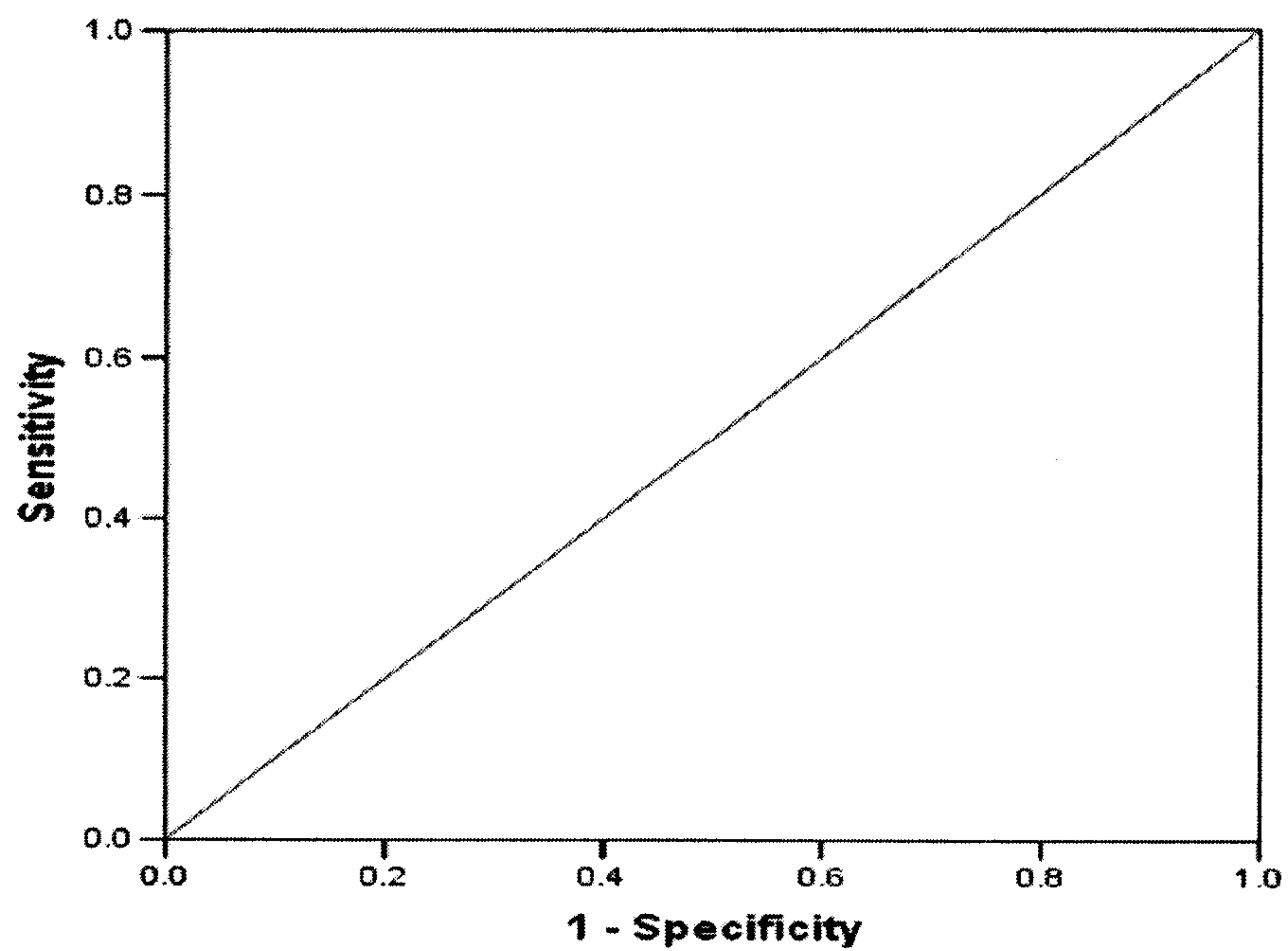


Figure 9c (cond.)

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Area Under the Curve

Test Result Variable(s): Probe 3

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
1.000	.000	.000	1.000	1.000

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 9c (cond.)

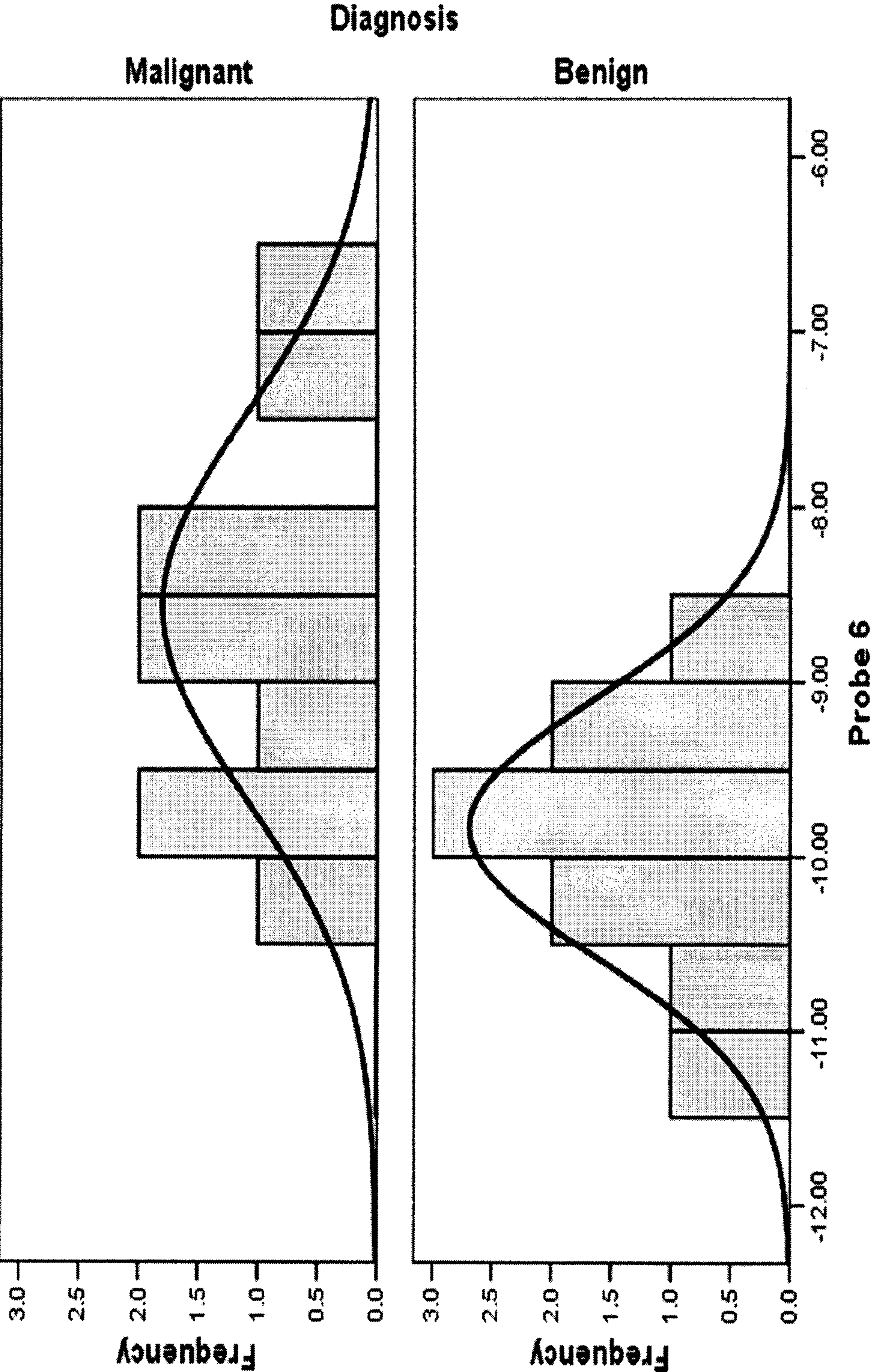


Figure 9d

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Coordinates of the Curve

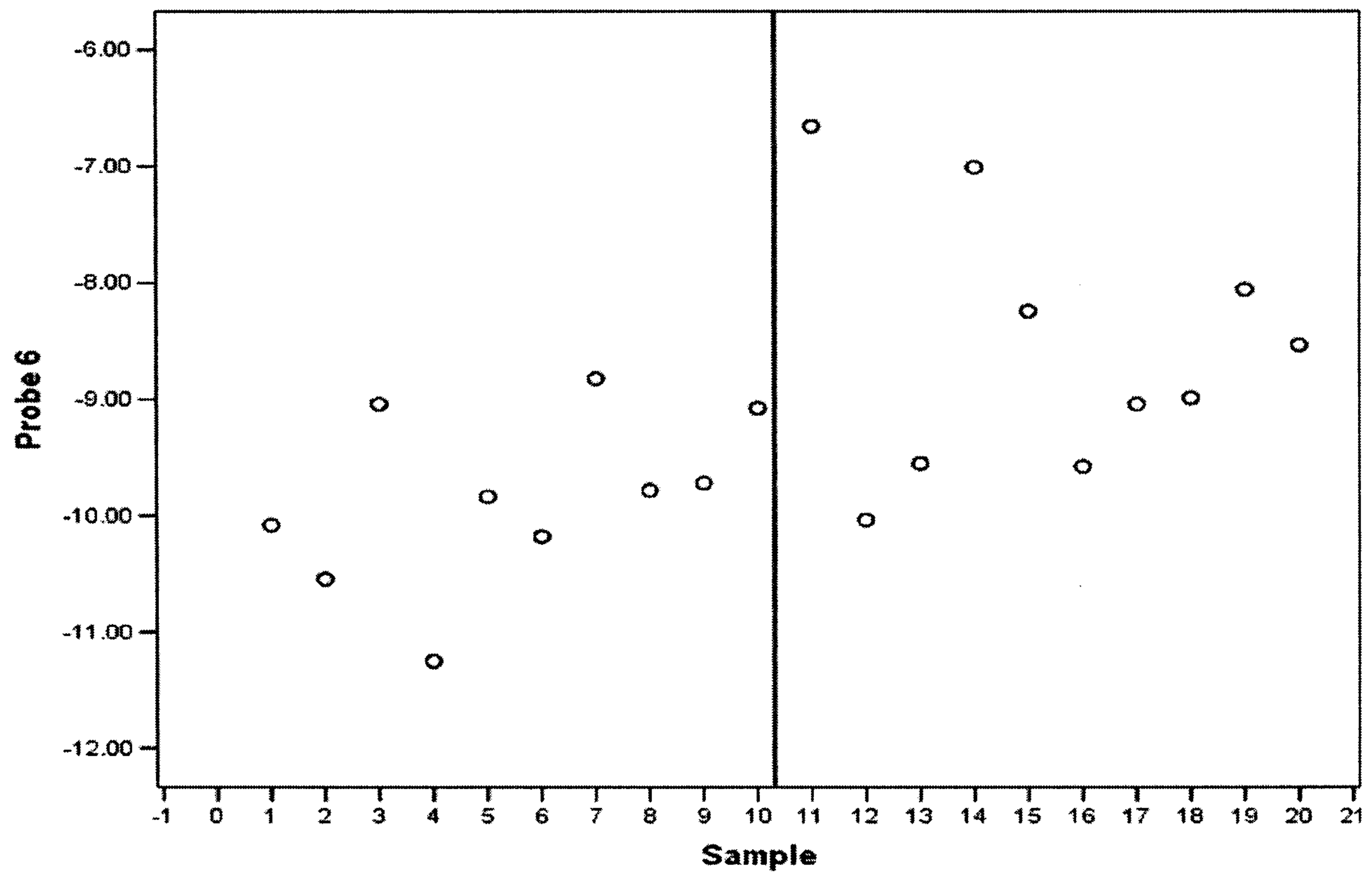
Test Result Variable(s): Probe 6

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-12.2484	1.000	1.000
-10.8974	1.000	.900
-10.3622	1.000	.800
-10.1290	1.000	.700
-10.0593	1.000	.600
-9.9364	.900	.600
-9.8080	.900	.500
-9.7497	.900	.400
-9.6479	.900	.300
-9.5650	.800	.300
-9.3142	.700	.300
-9.0593	.700	.200
-9.0419	.700	.100
-9.0155	.600	.100
-8.9061	.500	.100
-8.6798	.500	.000
-8.3909	.400	.000
-8.1522	.300	.000
-7.5330	.200	.000
-6.8311	.100	.000
-5.6551	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 9d (cond.)

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ROC Curve

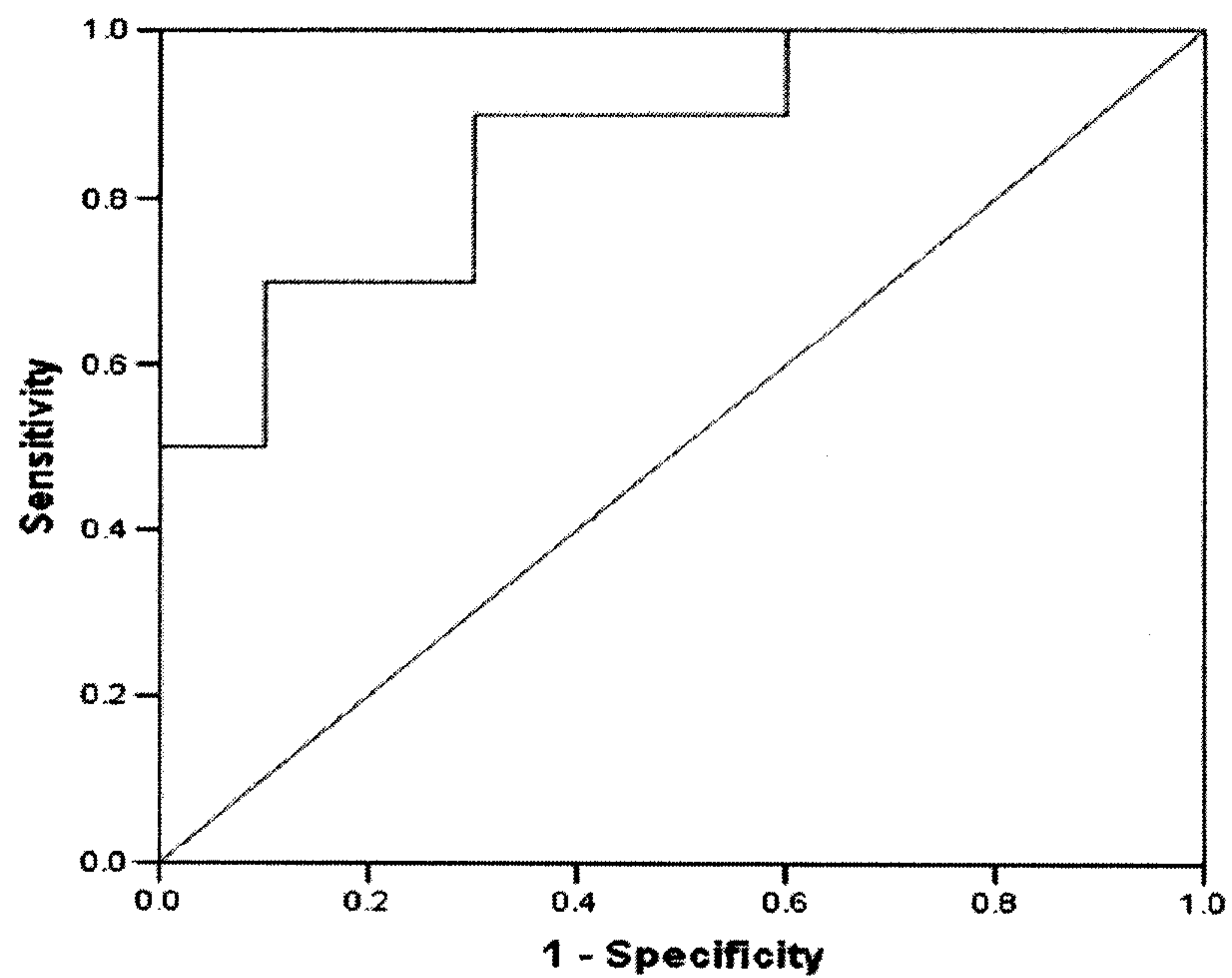


Figure 9d (cond.)

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Area Under the Curve

Test Result Variable(s): Probe 6

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.860	.083	.007	.698	1.022

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 9d (cond.)

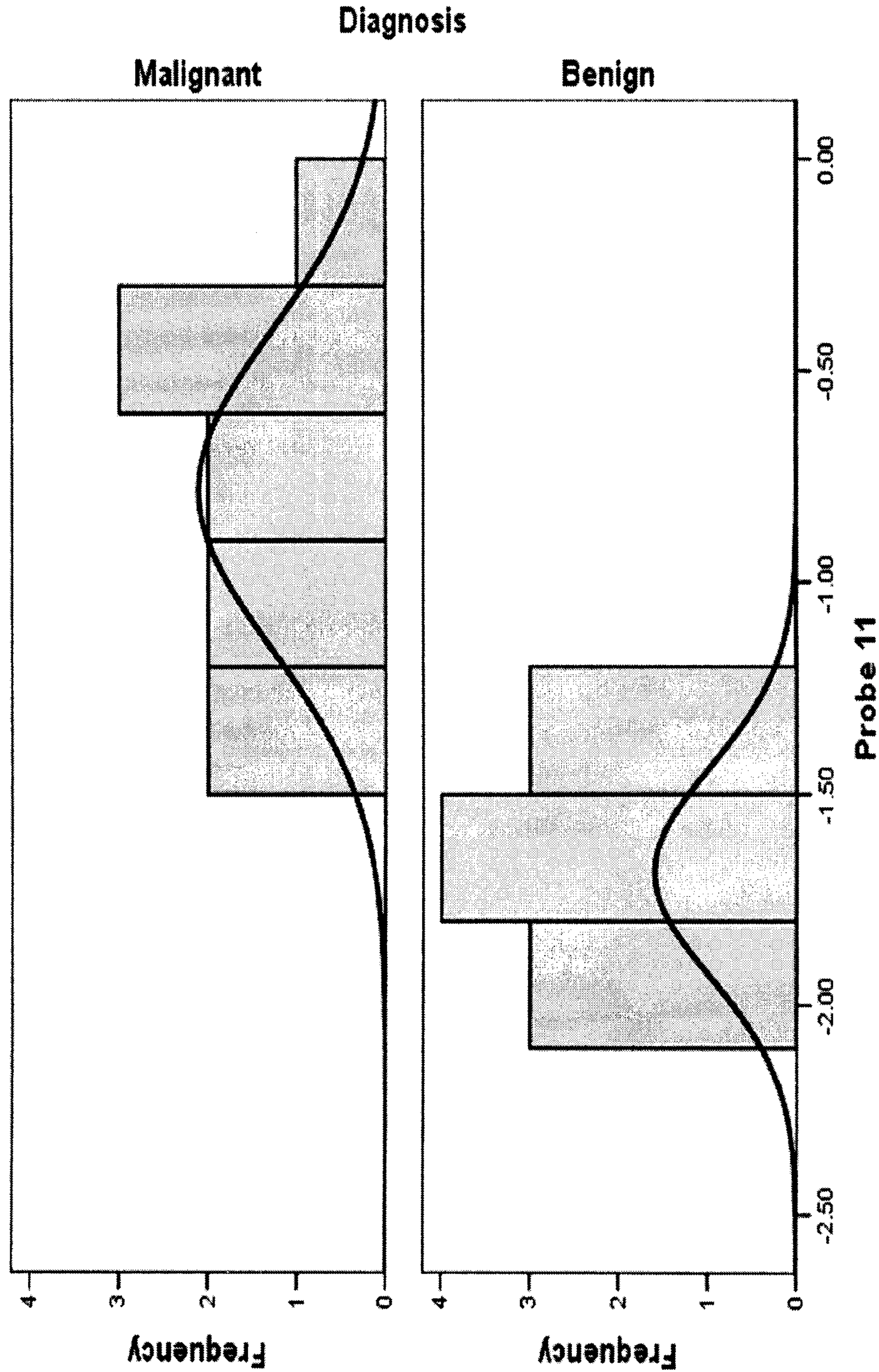


Figure 9e

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Coordinates of the Curve

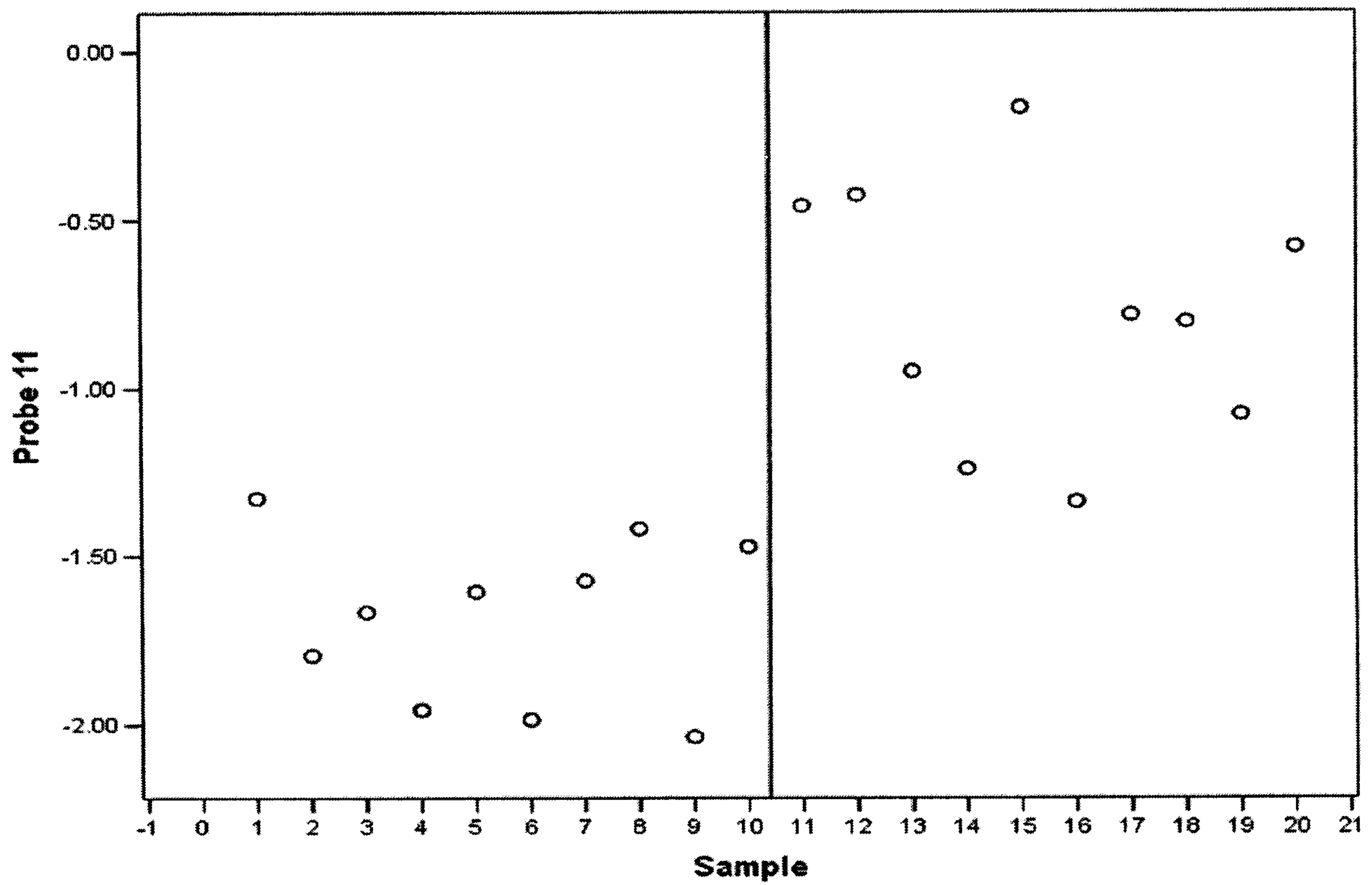
Test Result Variable(s): Probe 11

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-3.0352	1.000	1.000
-2.0097	1.000	.900
-1.9698	1.000	.800
-1.8754	1.000	.700
-1.7312	1.000	.600
-1.6369	1.000	.500
-1.5908	1.000	.400
-1.5241	1.000	.300
-1.4464	1.000	.200
-1.3794	1.000	.100
-1.3341	.900	.100
-1.2849	.900	.000
-1.1592	.800	.000
-1.0137	.700	.000
-.8767	.600	.000
-.7930	.500	.000
-.6813	.400	.000
-.5192	.300	.000
-.4421	.200	.000
-.2957	.100	.000
.8346	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 9e (cond.)

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ROC Curve

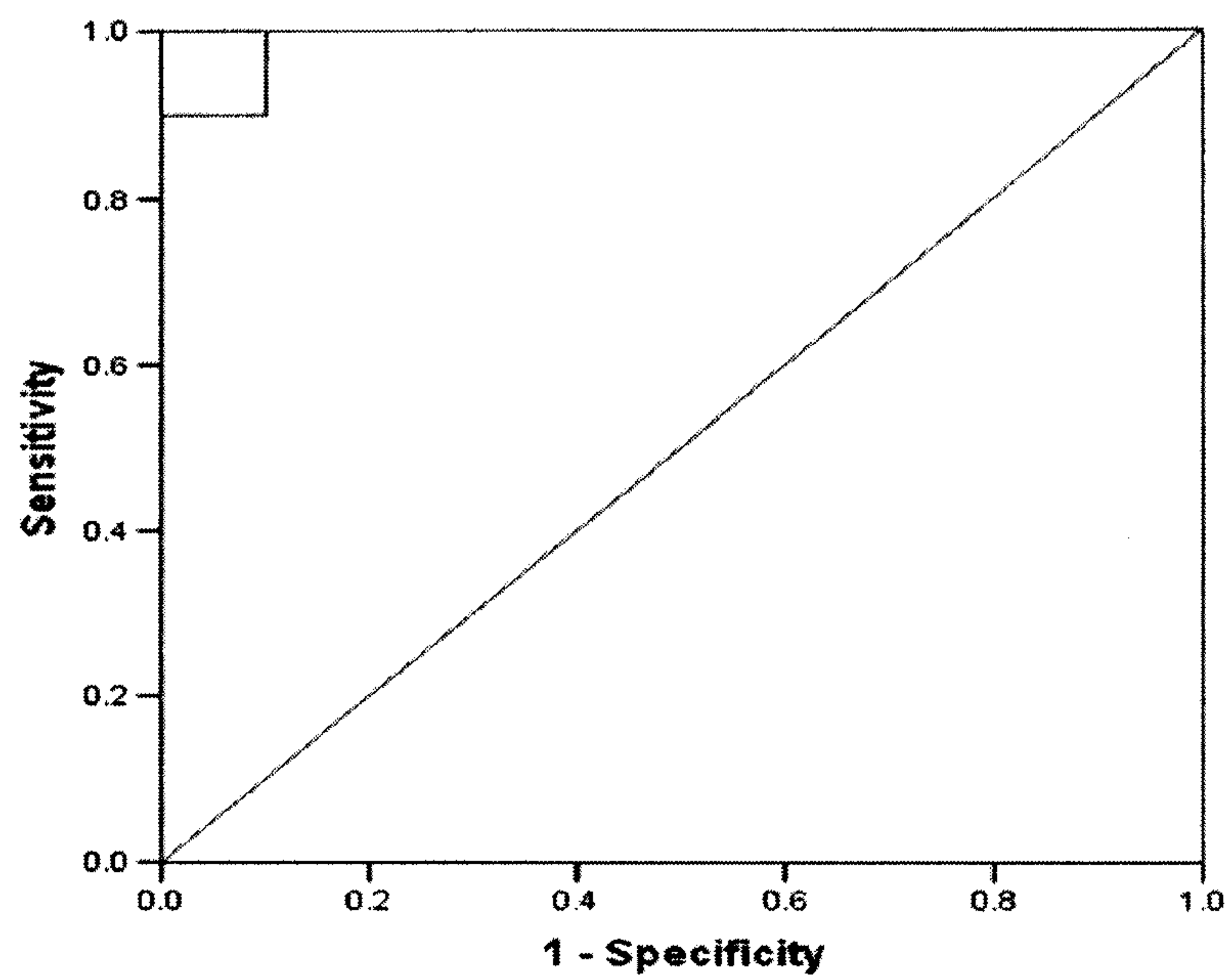


Figure 9e (cond.)

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Area Under the Curve

Test Result Variable(s): Probe 11

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.990	.016	.000	.958	1.022

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 9e (cond.)

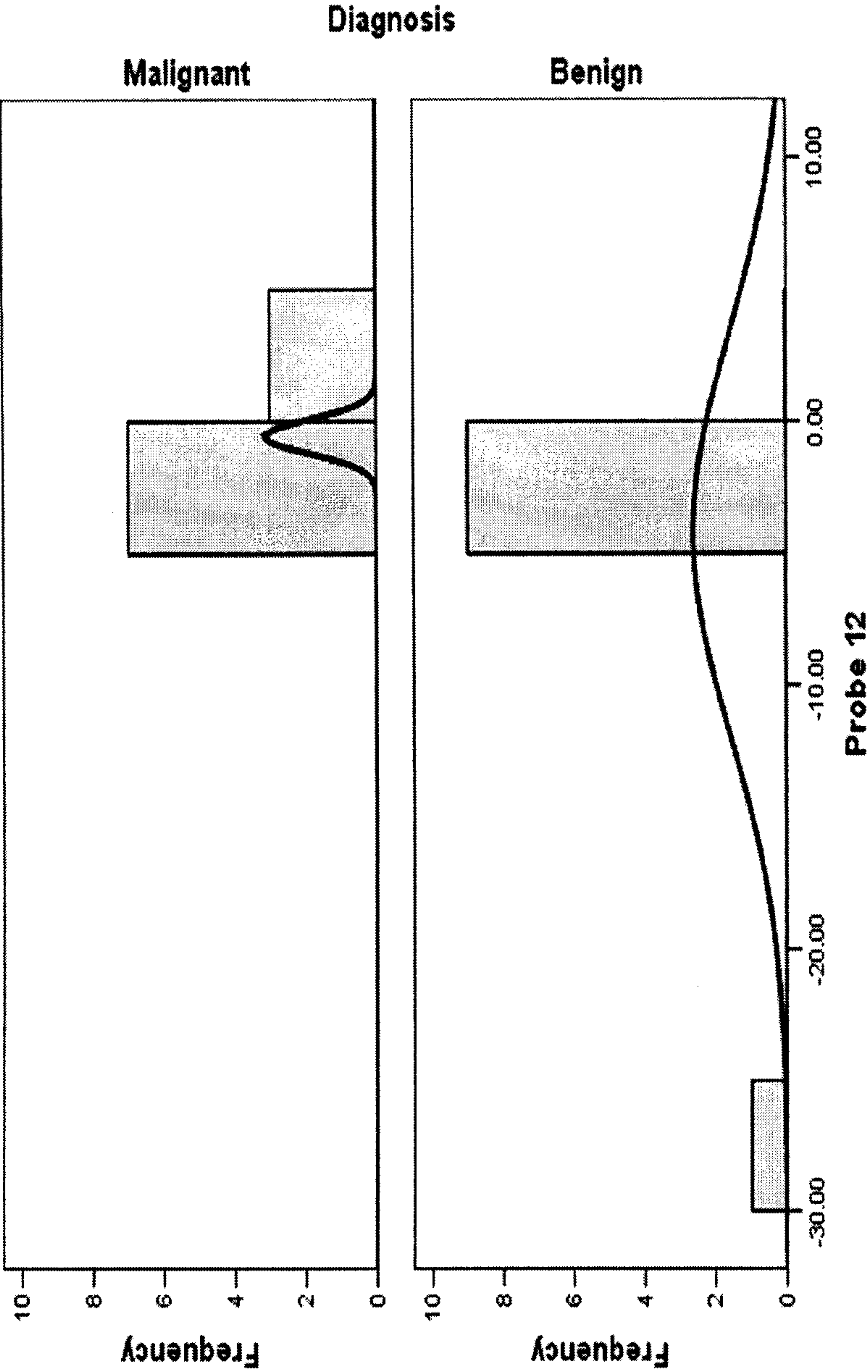


Figure 9f

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Coordinates of the Curve

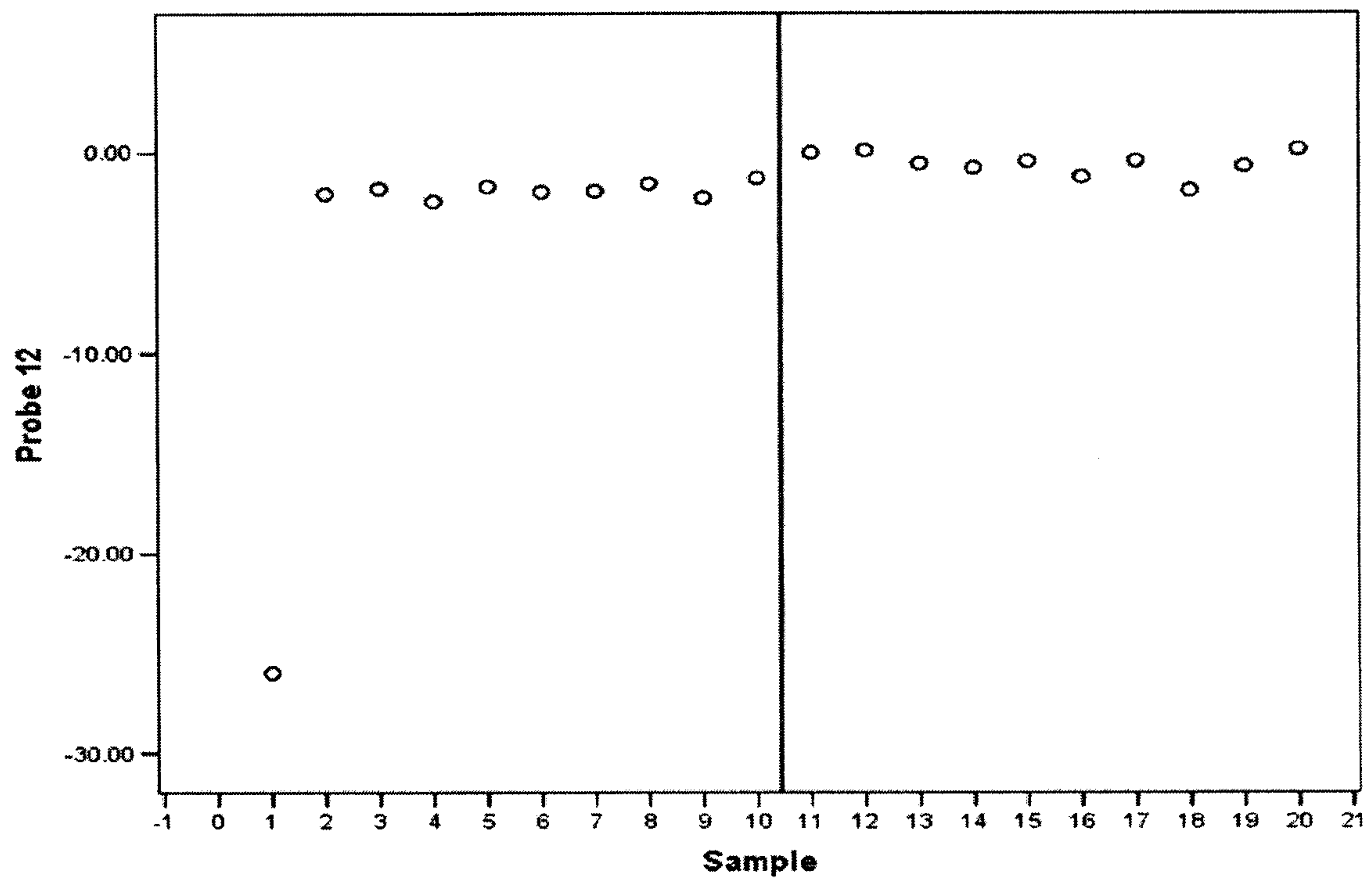
Test Result Variable(s): Probe 12

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-26.9813	1.000	1.000
-14.2108	1.000	.900
-2.3502	1.000	.800
-2.1570	1.000	.700
-2.0120	1.000	.600
-1.9490	1.000	.500
-1.9071	1.000	.400
-1.8435	.900	.400
-1.7488	.900	.300
-1.6219	.900	.200
-1.4090	.900	.100
-1.2379	.900	.000
-.9801	.800	.000
-.7077	.700	.000
-.6048	.600	.000
-.4856	.500	.000
-.4097	.400	.000
-.1985	.300	.000
.0613	.200	.000
.1524	.100	.000
1.1833	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 9f (cond.)

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ROC Curve

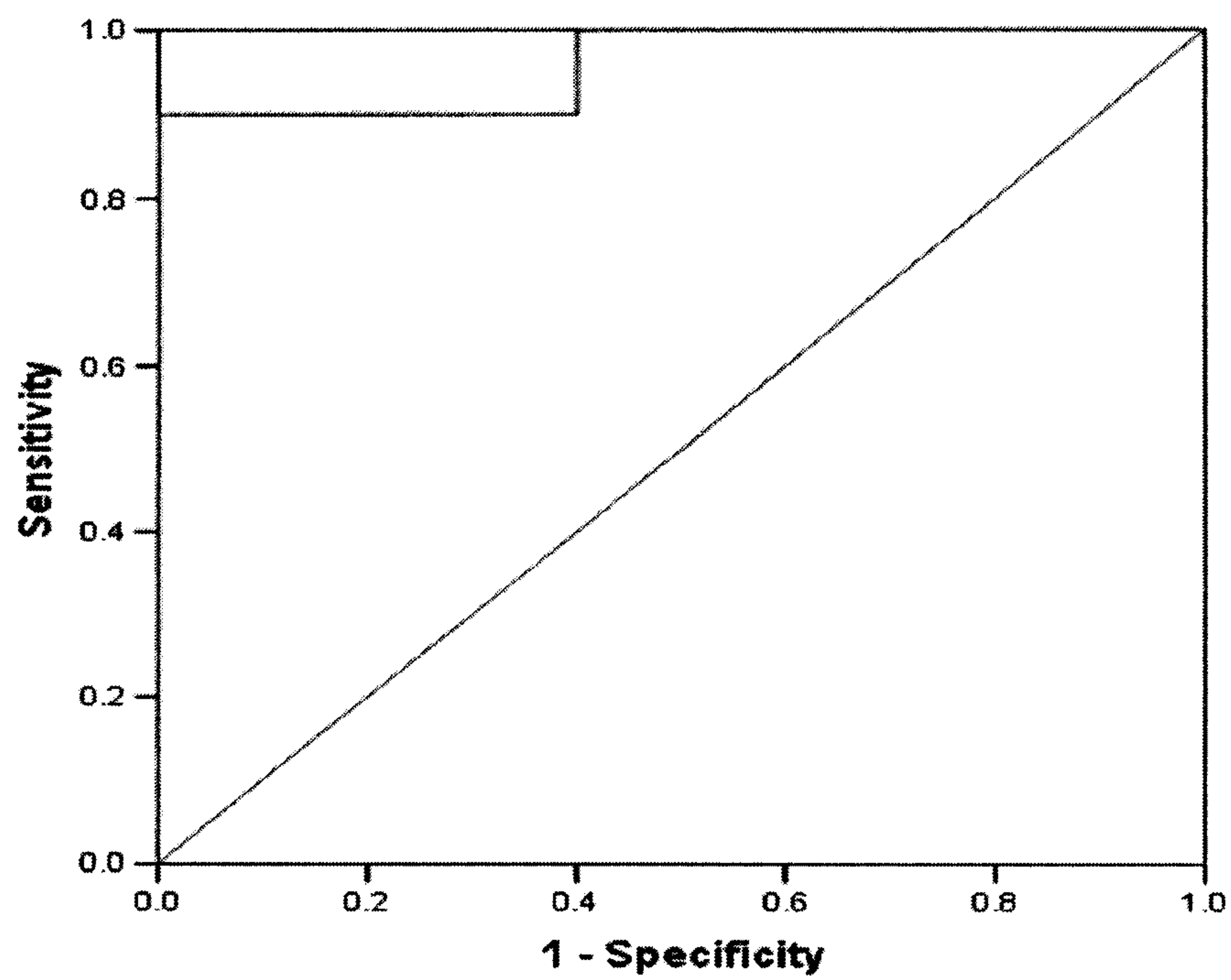


Figure 9f (cond.)

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Area Under the Curve

Test Result Variable(s): Probe 12

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.960	.044	.001	.875	1.045

- a. Under the nonparametric assumption
- b. Null hypothesis: true area = 0.5

Figure 9f (cond.)

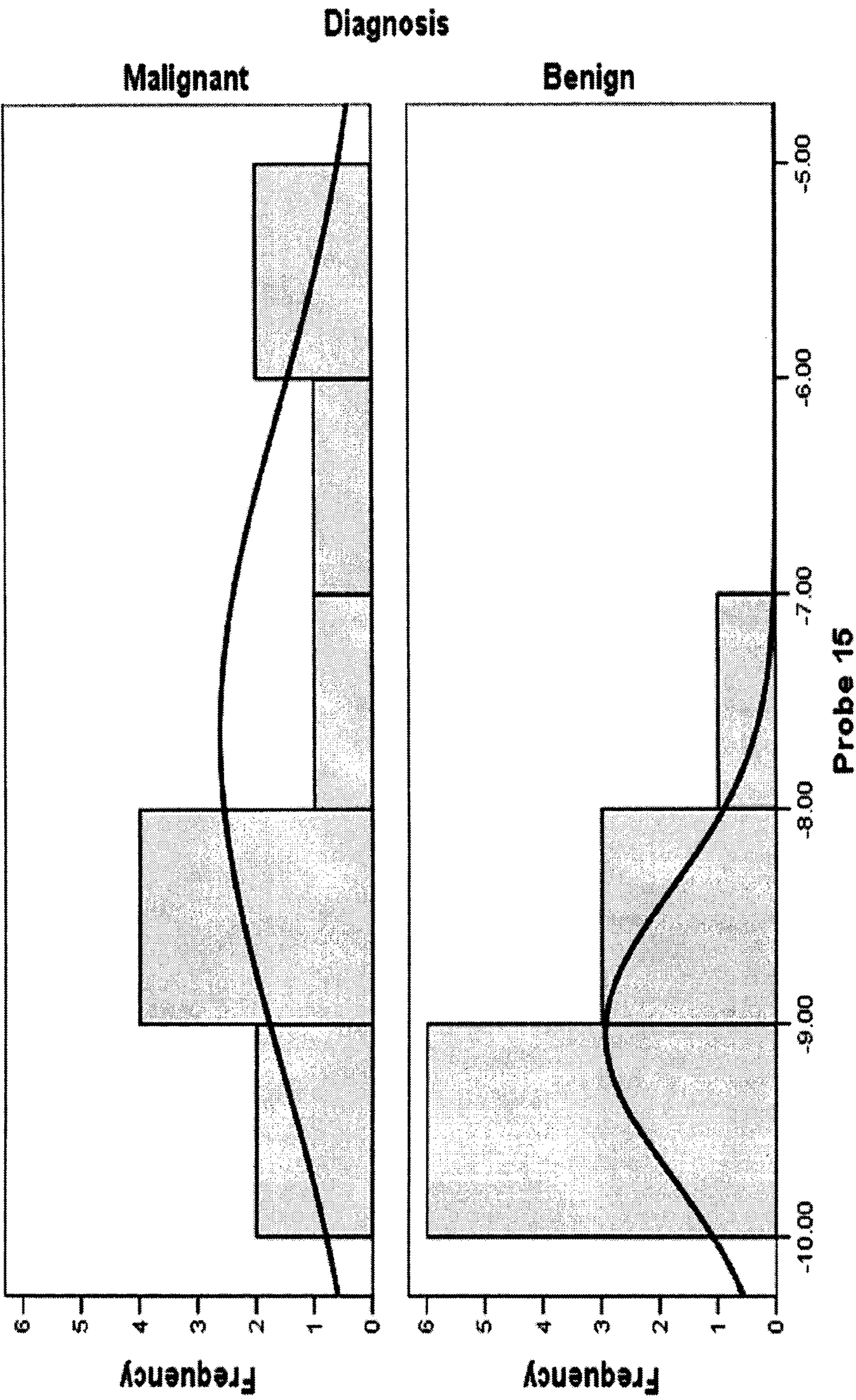


Figure 9g

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Coordinates of the Curve

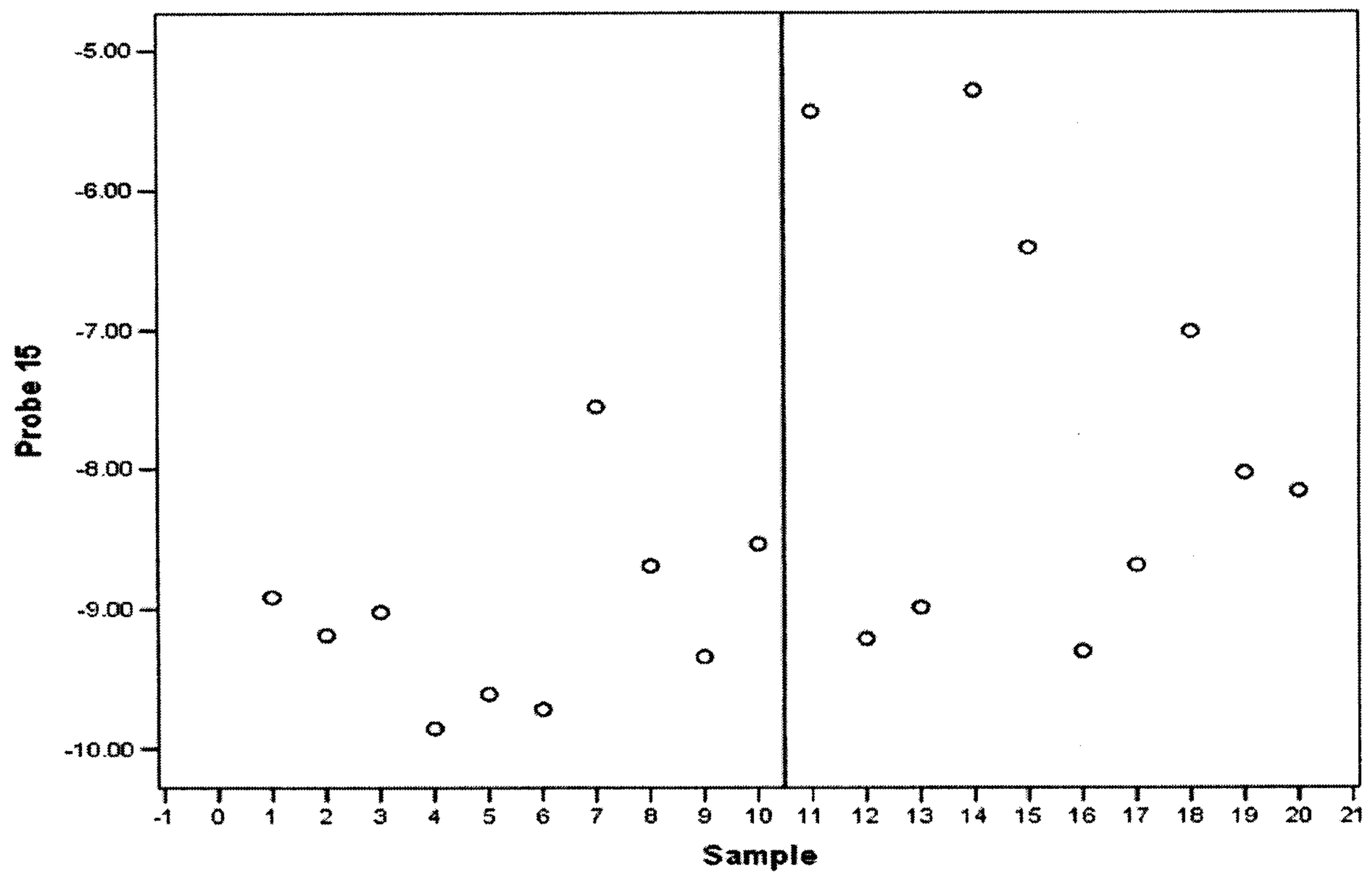
Test Result Variable(s): Probe 15

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-10.8536	1.000	1.000
-9.7839	1.000	.900
-9.6603	1.000	.800
-9.4725	1.000	.700
-9.3188	1.000	.600
-9.2553	.900	.600
-9.1992	.800	.600
-9.1050	.800	.500
-9.0054	.800	.400
-8.9524	.700	.400
-8.8066	.700	.300
-8.6926	.700	.200
-8.6143	.600	.200
-8.3481	.600	.100
-8.0930	.500	.100
-7.7916	.400	.100
-7.2842	.400	.000
-6.7136	.300	.000
-5.9261	.200	.000
-5.3645	.100	.000
-4.2896	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 9g (cond.)

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ROC Curve

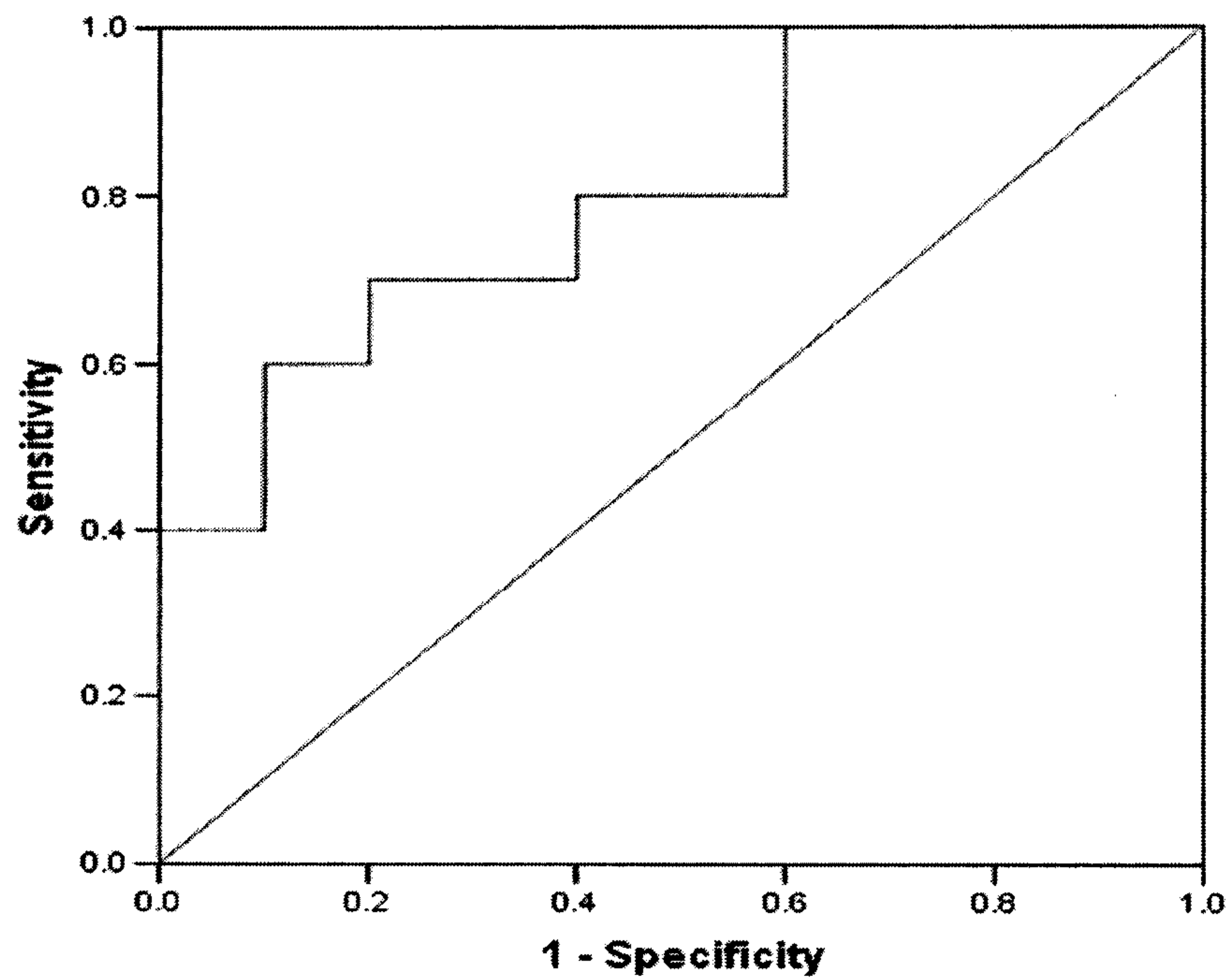


Figure 9g (cond.)

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Area Under the Curve

Test Result Variable(s): Probe 15

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.800	.099	.023	.605	.995

- a. Under the nonparametric assumption
- b. Null hypothesis: true area = 0.5

Figure 9g (cond.)

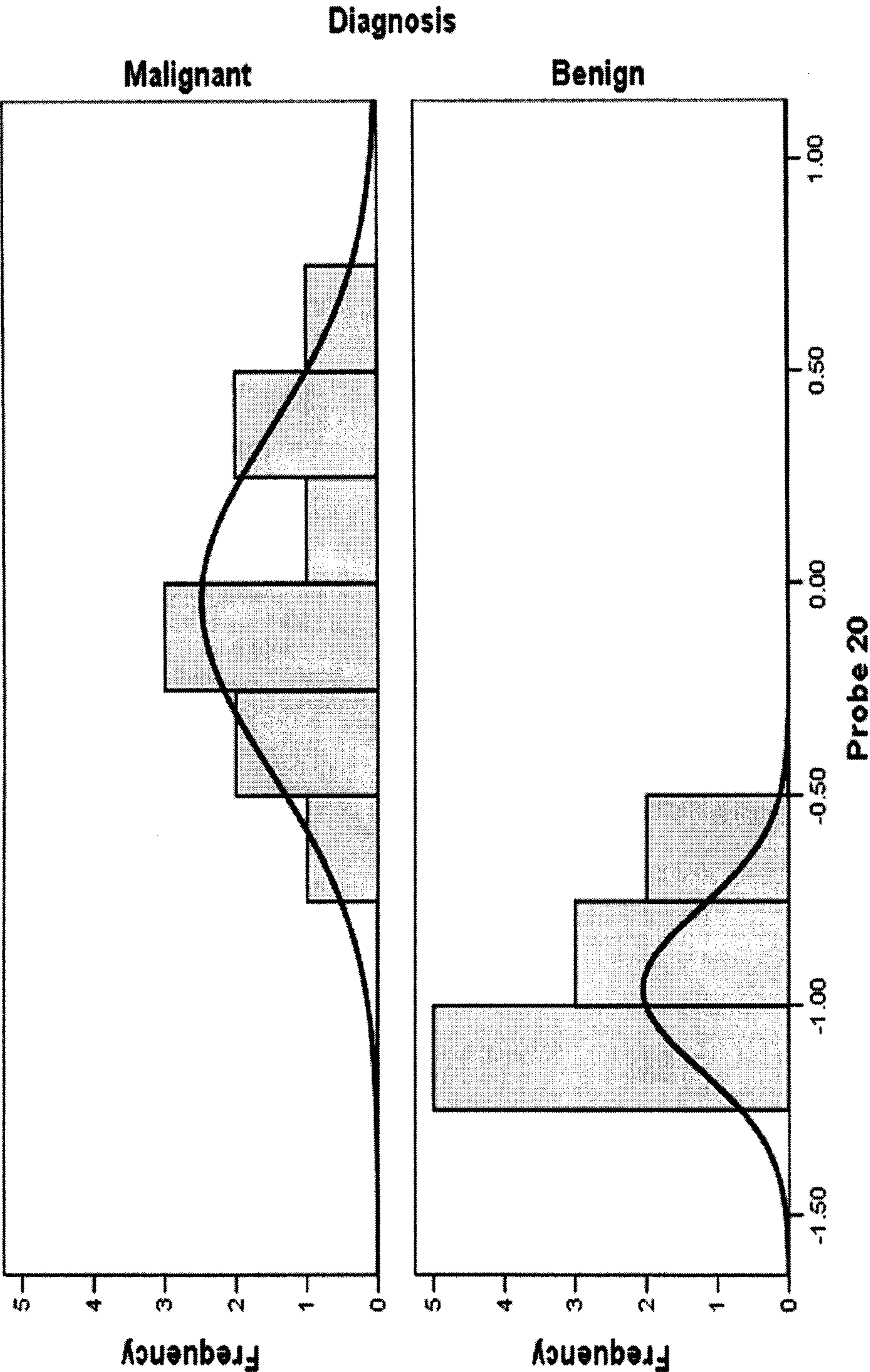


Figure 9h

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Coordinates of the Curve

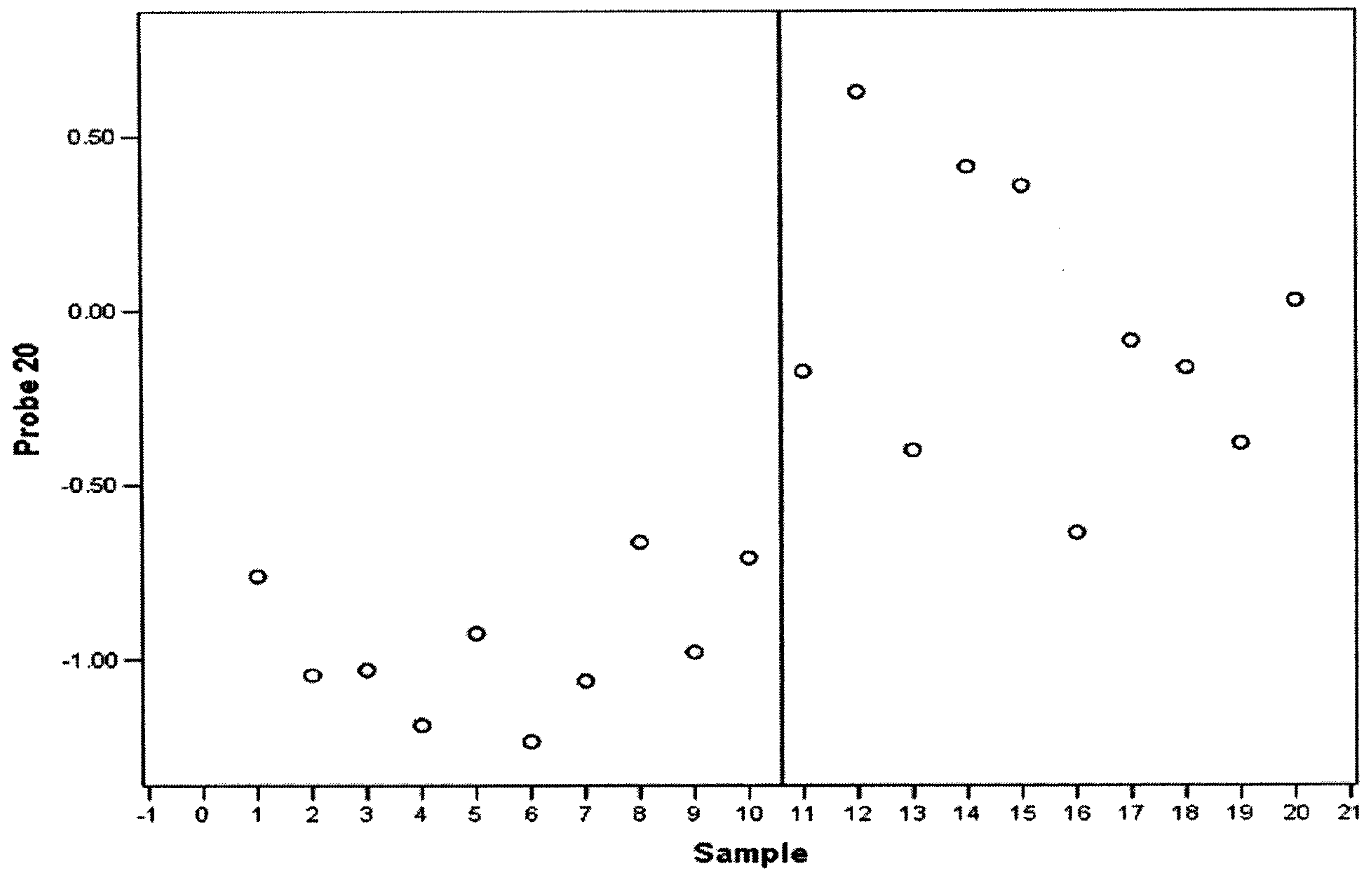
Test Result Variable(s): Probe 20

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-2.2352	1.000	1.000
-1.2122	1.000	.900
-1.1256	1.000	.800
-1.0534	1.000	.700
-1.0376	1.000	.600
-1.0051	1.000	.500
-.9534	1.000	.400
-.8448	1.000	.300
-.7367	1.000	.200
-.6881	1.000	.100
-.6521	1.000	.000
-.5198	.900	.000
-.3915	.800	.000
-.2781	.700	.000
-.1688	.600	.000
-.1252	.500	.000
-.0292	.400	.000
.1935	.300	.000
.3859	.200	.000
.5209	.100	.000
1.6286	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 9h (cond.)

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ROC Curve

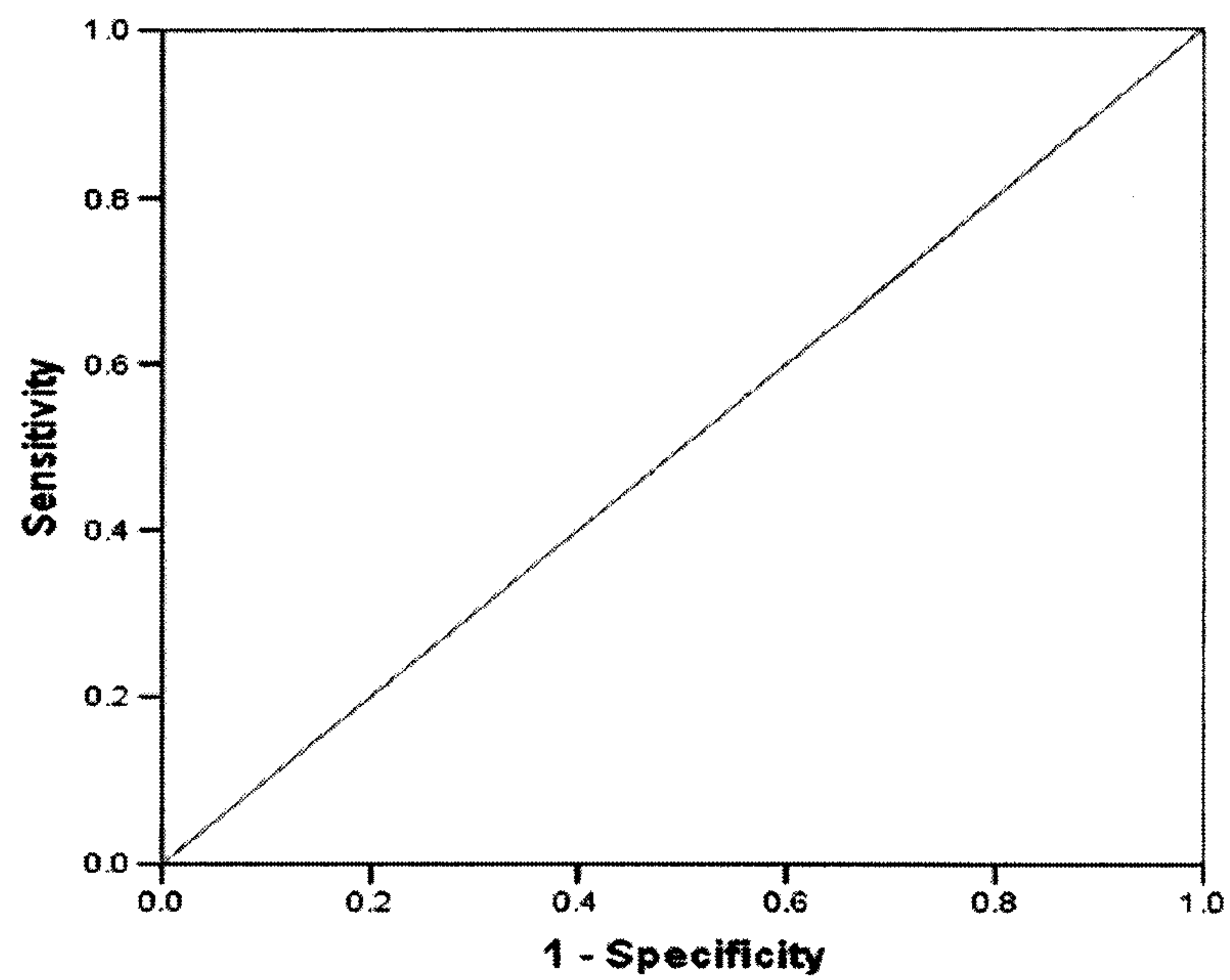


Figure 9h (cond.)

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Area Under the Curve

Test Result Variable(s): Probe 20

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
1.000	.000	.000	1.000	1.000

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 9h (cond.)

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Transcript 2

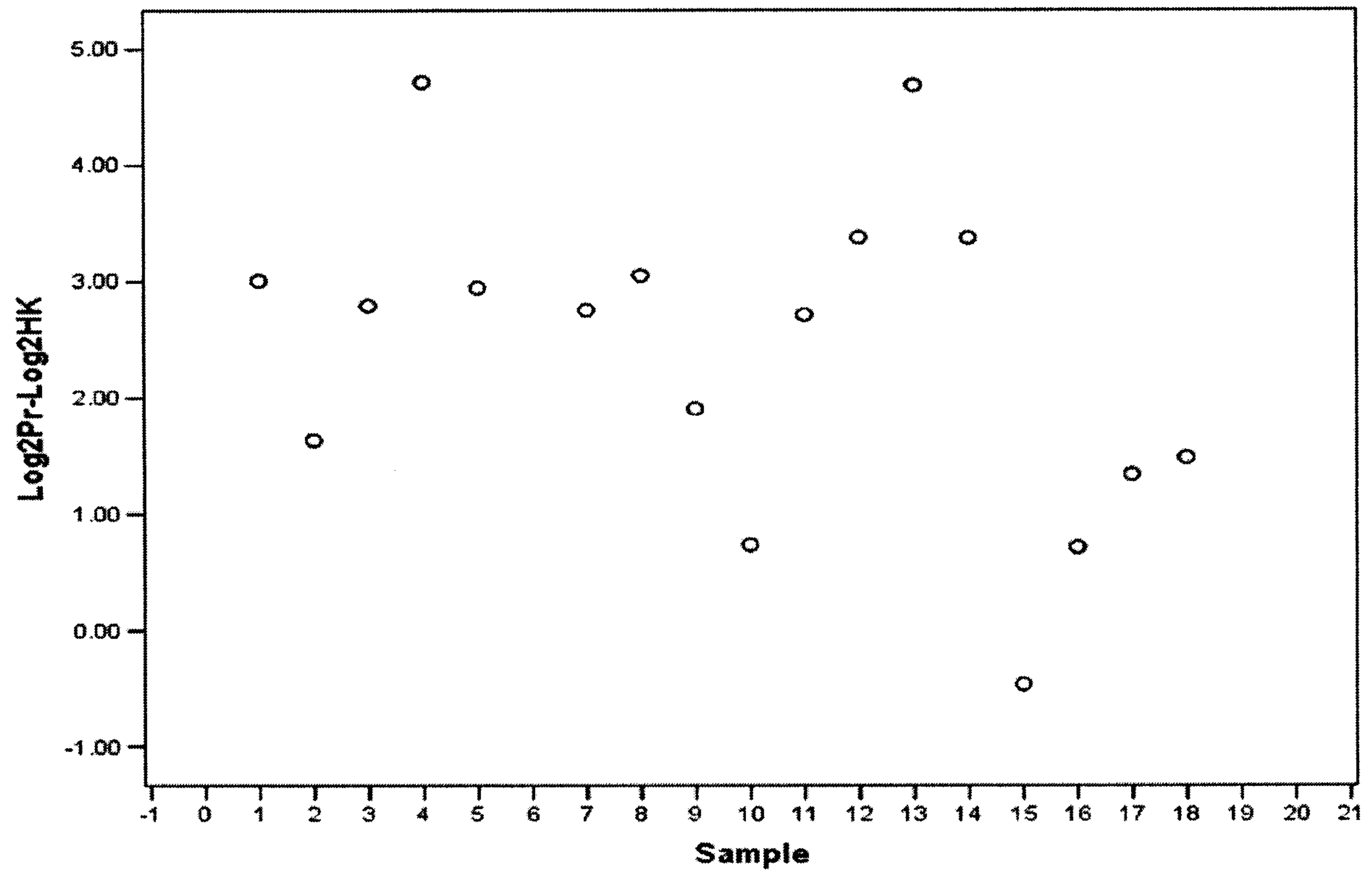


Figure 10a

Descriptives

Log2Pr-Log2HK		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean			Minimum	Maximum
						Lower Bound	Upper Bound			
Benign		8	2.8505	.92066	.32550	2.0808	3.6202		1.64	4.72
Non-Seminoma		5	2.9789	1.44319	.64542	1.1869	4.7708		.74	4.69
Seminoma		4	.7720	.88991	.44496	-.6440	2.1881		-.47	1.49
Total		17	2.3992	1.38160	.33509	1.6889	3.1096		-.47	4.72

Multiple Comparisons

Dependent Variable: Log2Pr-Log2HK
Tukey HSD

(I) Secondary Diagnosis	(J) Secondary Diagnosis	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Benign	Non-Seminoma	-.12836	.62153	.977	-1.7551	1.4983
	Seminoma	2.07851*	.66763	.020	.3311	3.8259
Non-Seminoma	Benign	.12836	.62153	.977	-1.4983	1.7551
	Seminoma	2.20686*	.73135	.024	.2927	4.1210
Seminoma	Benign	-2.07851*	.66763	.020	-3.8259	-.3311
	Non-Seminoma	-2.20686*	.73135	.024	-4.1210	-.2927

*. The mean difference is significant at the .05 level.

Figure 10a (cond.)

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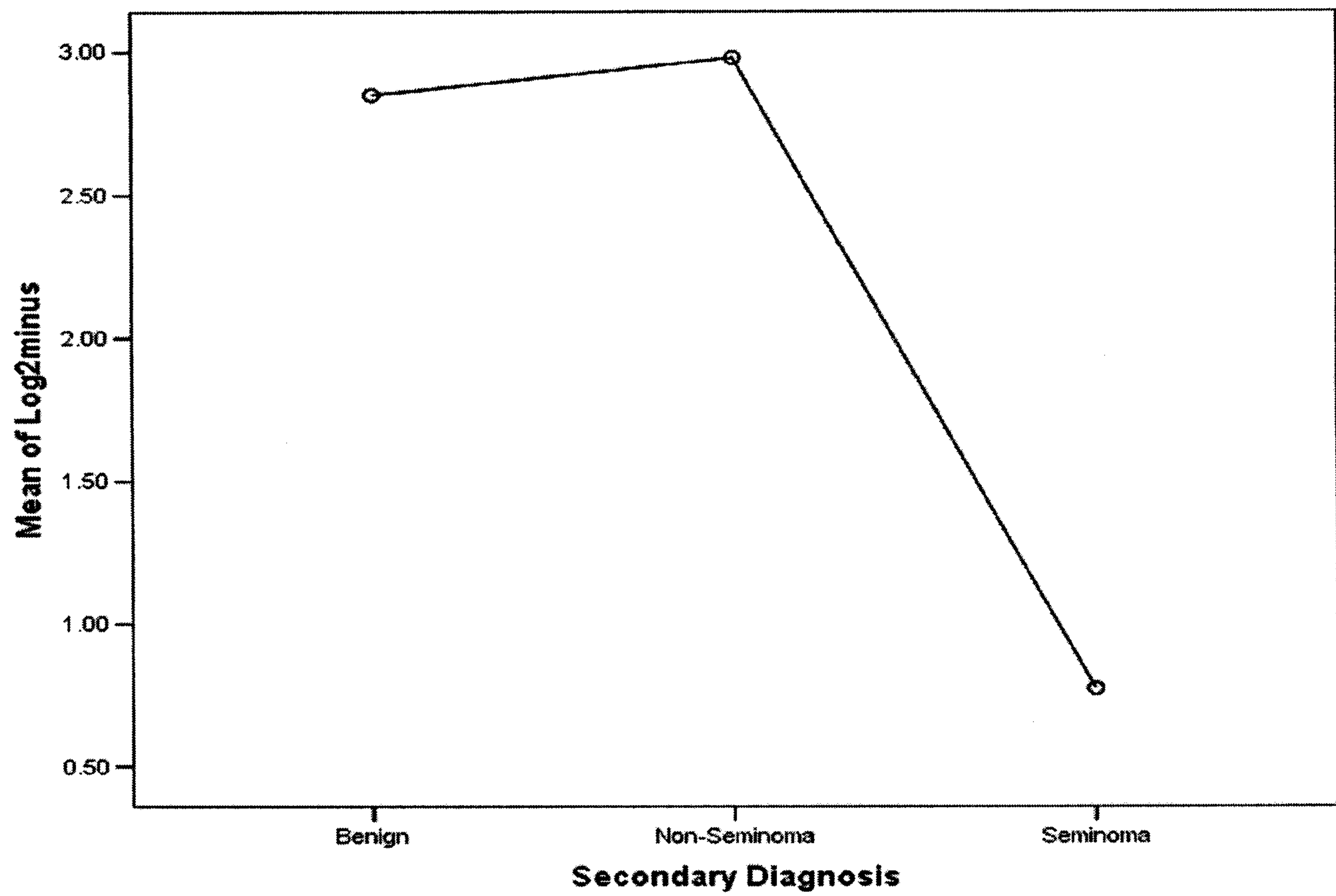
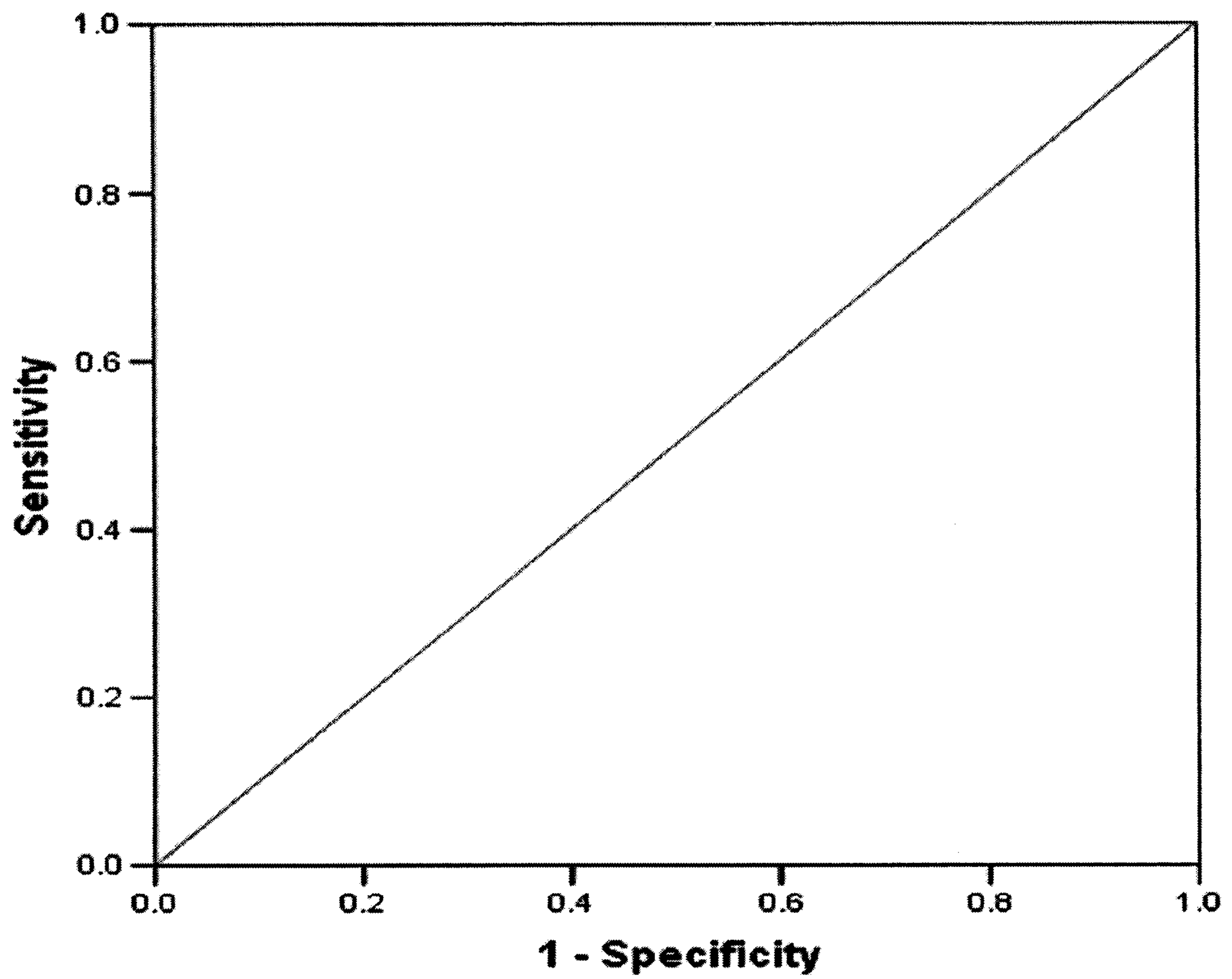


Figure 10a (cond.)

76/133

Benign to Seminoma**ROC Curve****Figure 10b**

77/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-1.4662	.000	.000
.1280	.250	.000
1.0328	.500	.000
1.4161	.750	.000
1.5621	1.000	.000
1.7712	1.000	.125
2.3297	1.000	.250
2.7718	1.000	.375
2.8684	1.000	.500
2.9759	1.000	.625
3.0282	1.000	.750
3.8831	1.000	.875
5.7161	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
1.000	.000	.007	1.000	1.000

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 10b (cond.)

78/133

Non-Seminoma to Seminoma

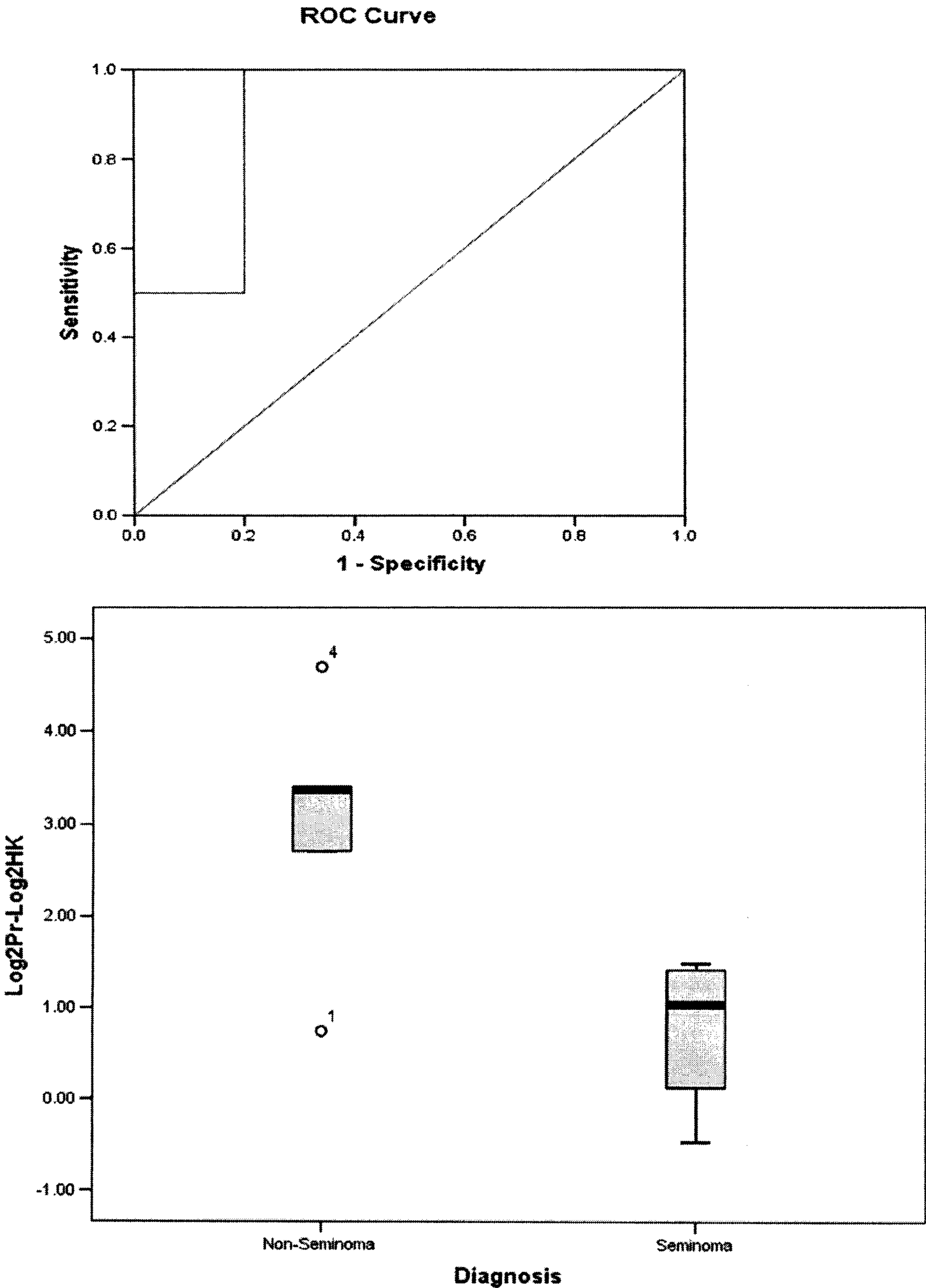


Figure 10b (cond.)

79/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-1.4662	.000	.000
.1280	.250	.000
.7309	.500	.000
1.0415	.500	.200
1.4161	.750	.200
2.1006	1.000	.200
3.0434	1.000	.400
3.3757	1.000	.600
4.0340	1.000	.800
5.6909	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

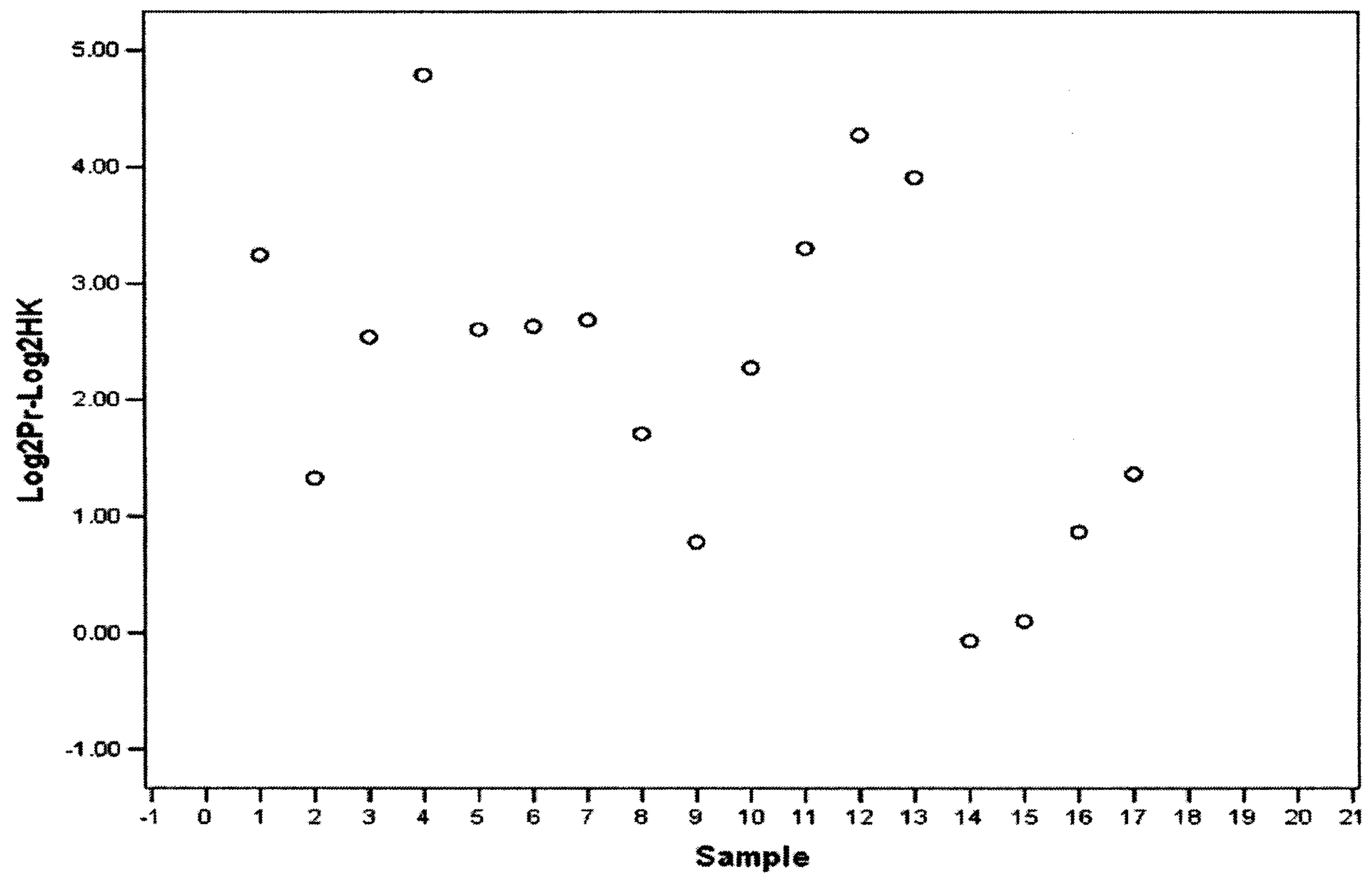
Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.900	.112	.050	.681	1.119

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 10b (cond.)

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Transcript 3**Figure 11a**

Descriptives

Log2Pr-Log2HK		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Benign		8	2.6917	1.04169	.36829	1.8208	3.5625	1.33	4.79
Non-Seminoma		5	2.9100	1.41243	.63166	1.1562	4.6638	.78	4.28
Seminoma		4	.5670	.67029	.33515	-.4996	1.6336	-.07	1.36
Total		17	2.2560	1.41401	.34295	1.5289	2.9830	-.07	4.79

Figure 11a (cond.)

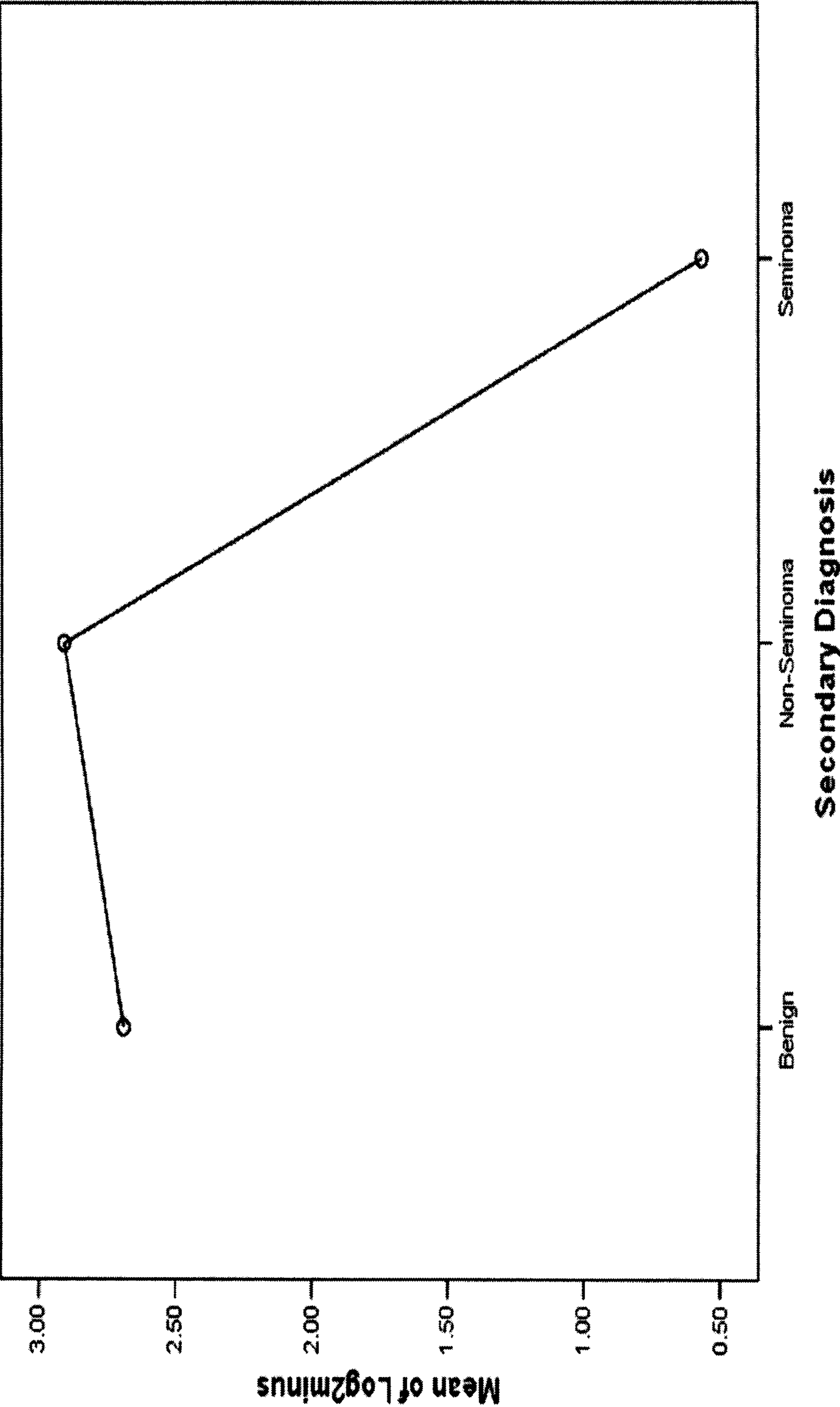
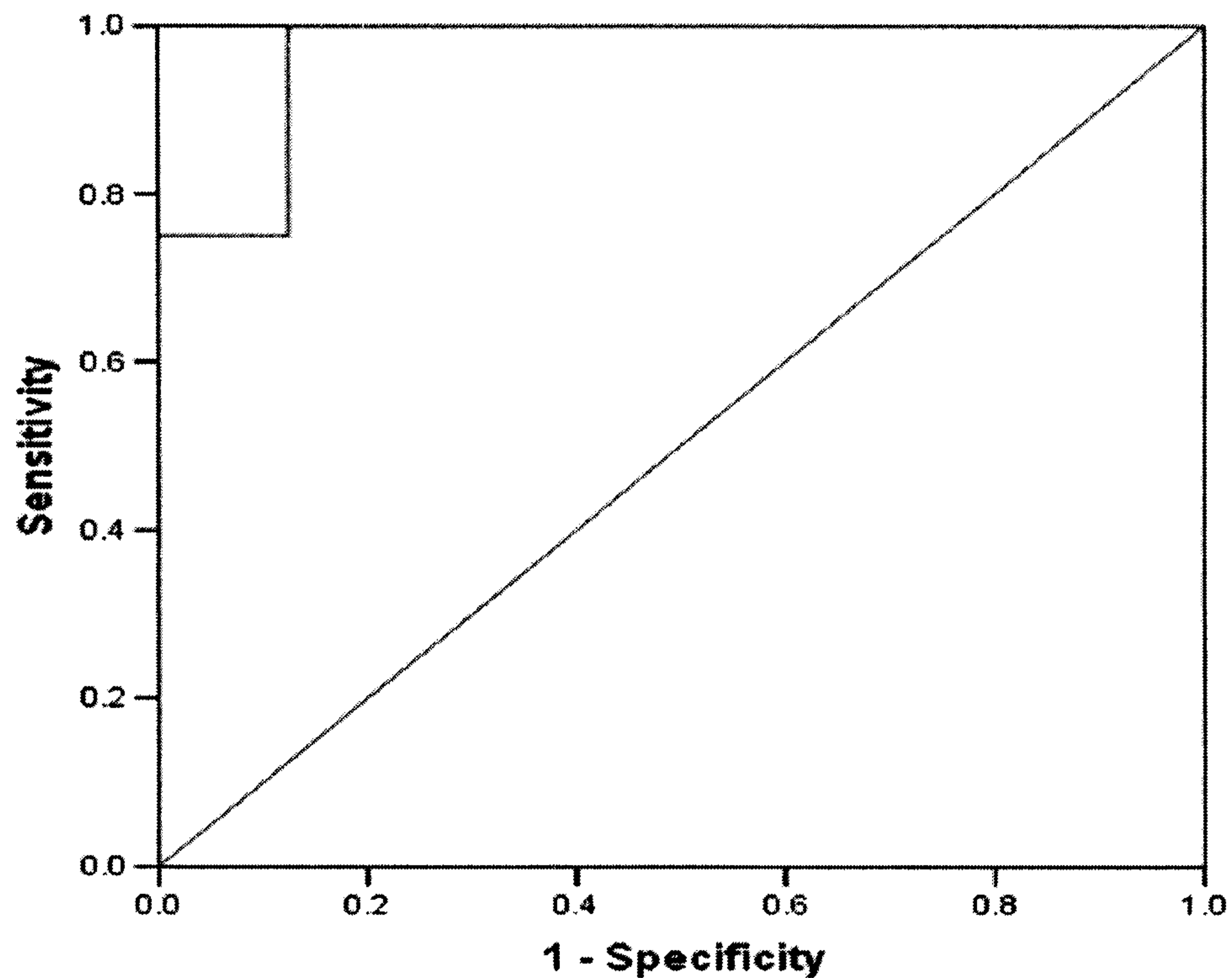


Figure 11a (cond.)

83/133

Benign to Seminoma**ROC Curve****Coordinates of the Curve**

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-1.0665	.000	.000
.0167	.250	.000
.4848	.500	.000
1.0981	.750	.000
1.3457	.750	.125
1.5359	1.000	.125
2.1229	1.000	.250
2.5712	1.000	.375
2.6177	1.000	.500
2.6580	1.000	.625
2.9661	1.000	.750
4.0208	1.000	.875
5.7935	1.000	1.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 11b

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Area Under the Curve

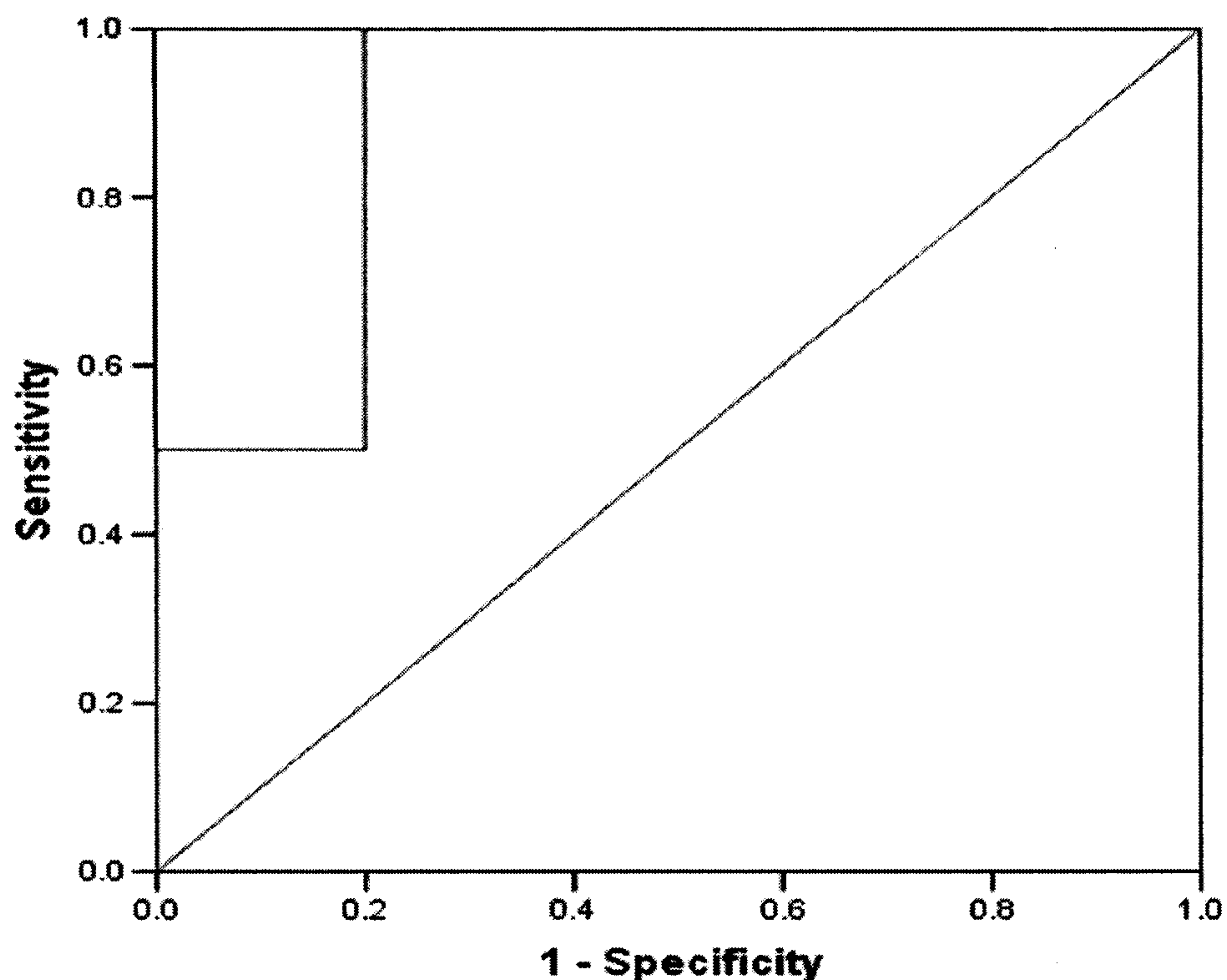
Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.969	.047	.011	.876	1.061

- a. Under the nonparametric assumption
- b. Null hypothesis: true area = 0.5

Figure 11b (cond.)

85/133

Non-Seminoma to Seminoma**ROC Curve****Coordinates of the Curve**

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-1.0665	.000	.000
.0167	.250	.000
.4403	.500	.000
.8251	.500	.200
1.1173	.750	.200
1.8181	1.000	.200
2.7884	1.000	.400
3.6099	1.000	.600
4.0963	1.000	.800
5.2783	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Figure 11b (cond.)

86/133

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

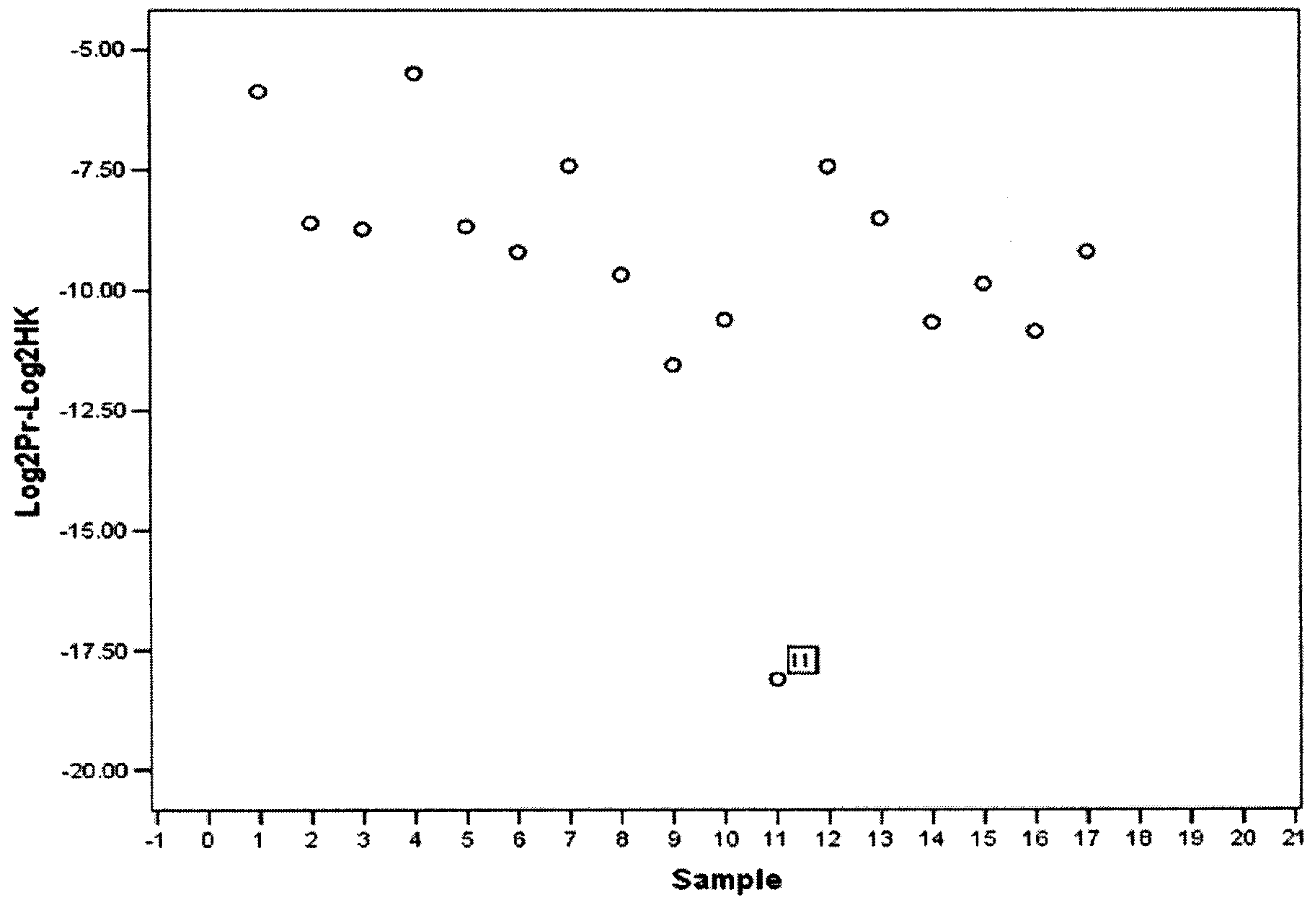
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.900	.112	.050	.681	1.119

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 11b (cond.)

87/133

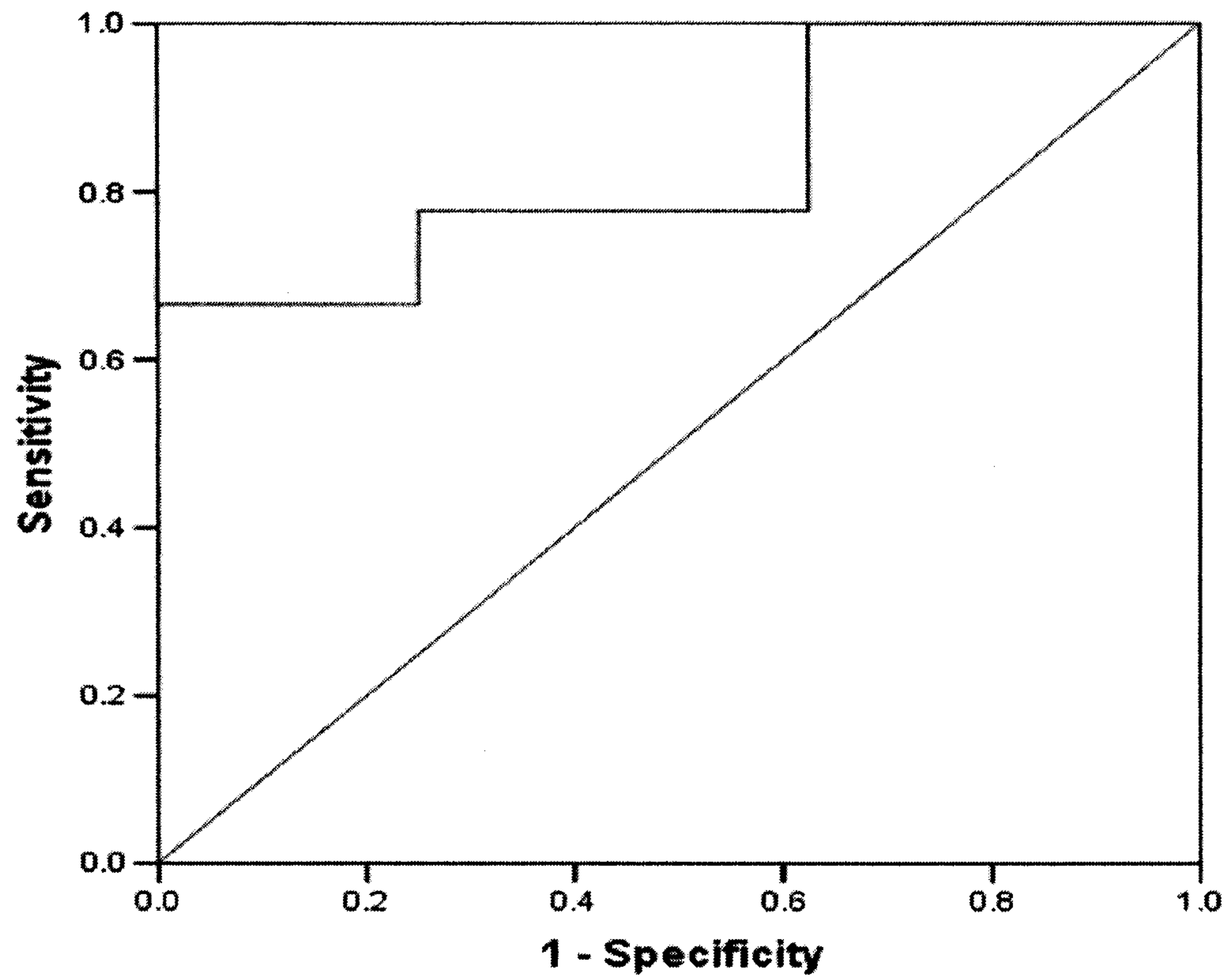
Transcript 4**Figure 12**

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Log2Pr-Log2HK	.424	.525	2.337	15	.034	2.79398	1.19570	.24540	5.34256
			2.425	12.142	.032	2.79398	1.15230	.28660	5.30136

Figure 12 (cond.)

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ROC Curve**Figure 12 (cond.)**

90/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-19.1032	.000	.000
-14.8271	.111	.000
-11.1973	.222	.000
-10.7498	.333	.000
-10.6312	.444	.000
-10.2319	.556	.000
-9.7628	.667	.000
-9.4346	.667	.125
-9.1925	.667	.250
-8.9503	.778	.250
-8.6923	.778	.375
-8.6324	.778	.500
-8.5458	.778	.625
-7.9611	.889	.625
-7.4208	1.000	.625
-6.6410	1.000	.750
-5.6799	1.000	.875
-4.4918	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.833	.102	.021	.633	1.033

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 12 (cond.)

91/133

Transcript 11

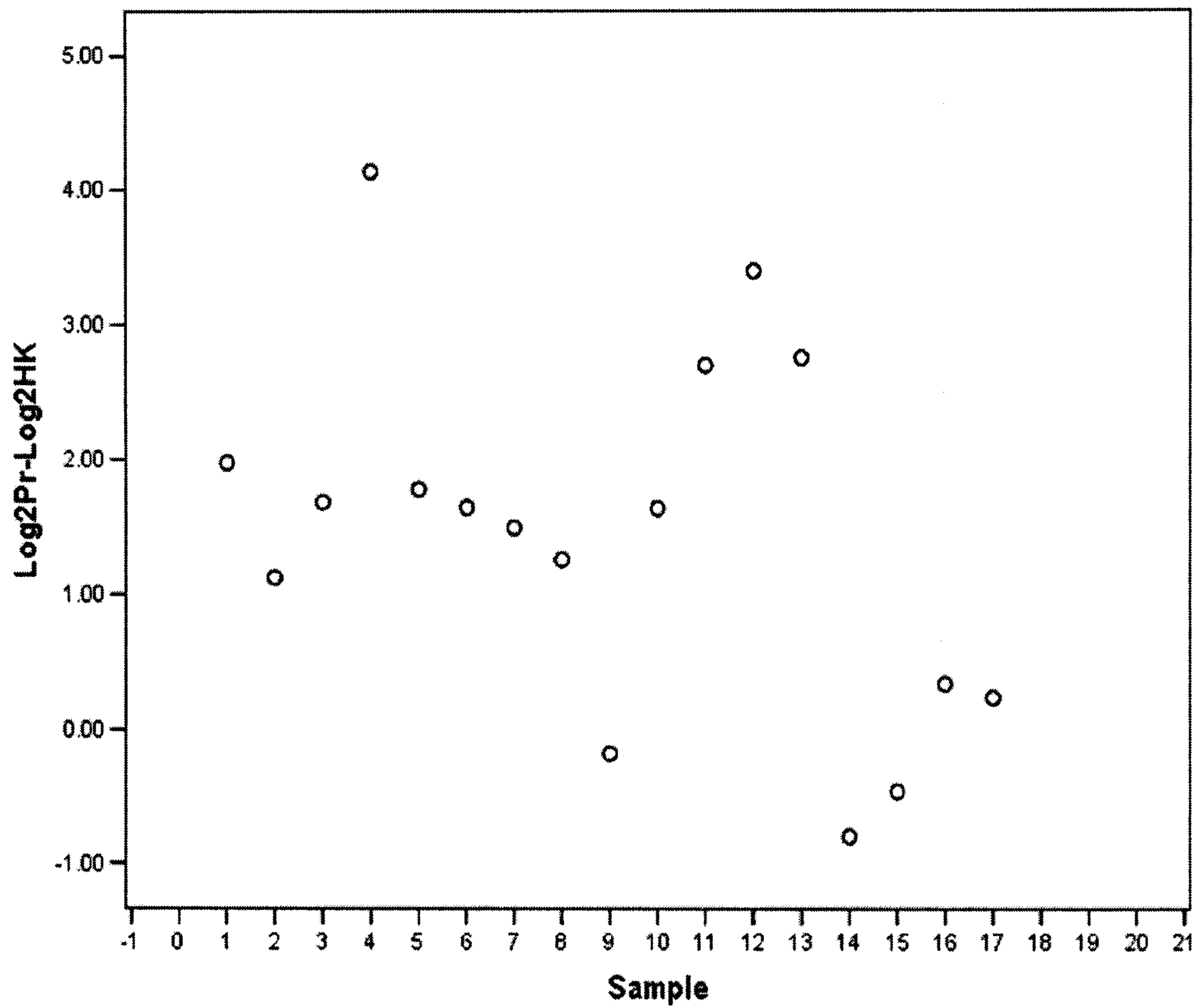


Figure 13a

Multiple Comparisons

Dependent Variable: Log2P_r-Log2HK
Tukey HSD

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
(I) Secondary Diagnosis	(J) Secondary Diagnosis					
	Benign					
	Non-Seminoma	-.17586	.59248	.953	-1.7265	1.3748
	Seminoma	2.05715*	.63643	.016	.3914	3.7229
Non-Seminoma	Benign	.17586	.59248	.953	-1.3748	1.7265
	Seminoma	2.23301*	.69717	.016	.4083	4.0577
Seminoma	Benign	-2.05715*	.63643	.016	-3.7229	-.3914
	Non-Seminoma	-2.23301*	.69717	.016	-4.0577	-.4083

*. The mean difference is significant at the .05 level.

Figure 13a (cond.)

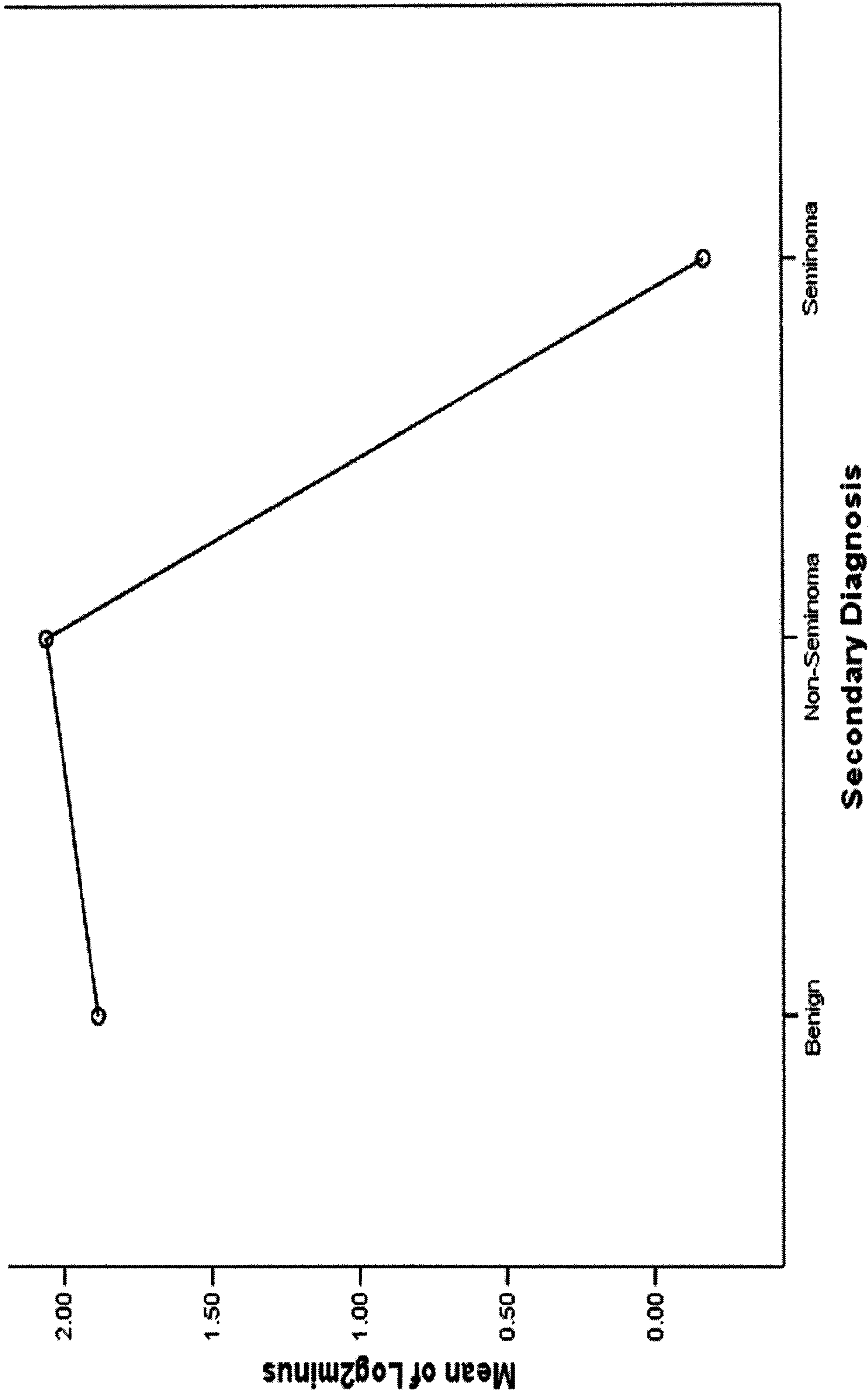
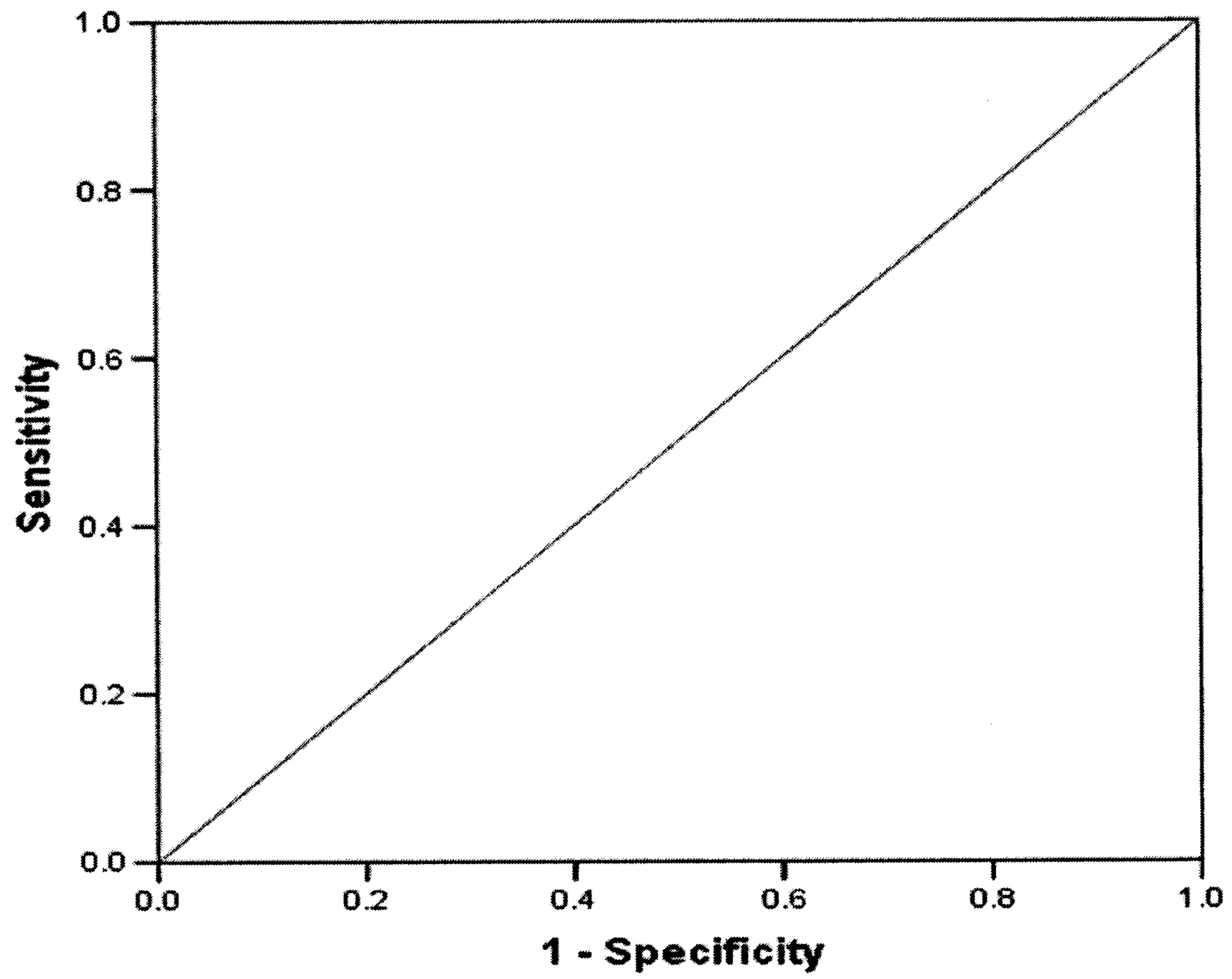


Figure 13a (cond.)

94/133

Benign to Seminoma**ROC Curve****Figure 13b**

95/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-1.7974	.000	.000
-.6304	.250	.000
-.1127	.500	.000
.2892	.750	.000
.7320	1.000	.000
1.1910	1.000	.125
1.3755	1.000	.250
1.5677	1.000	.375
1.6627	1.000	.500
1.7308	1.000	.625
1.8766	1.000	.750
3.0567	1.000	.875
5.1393	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

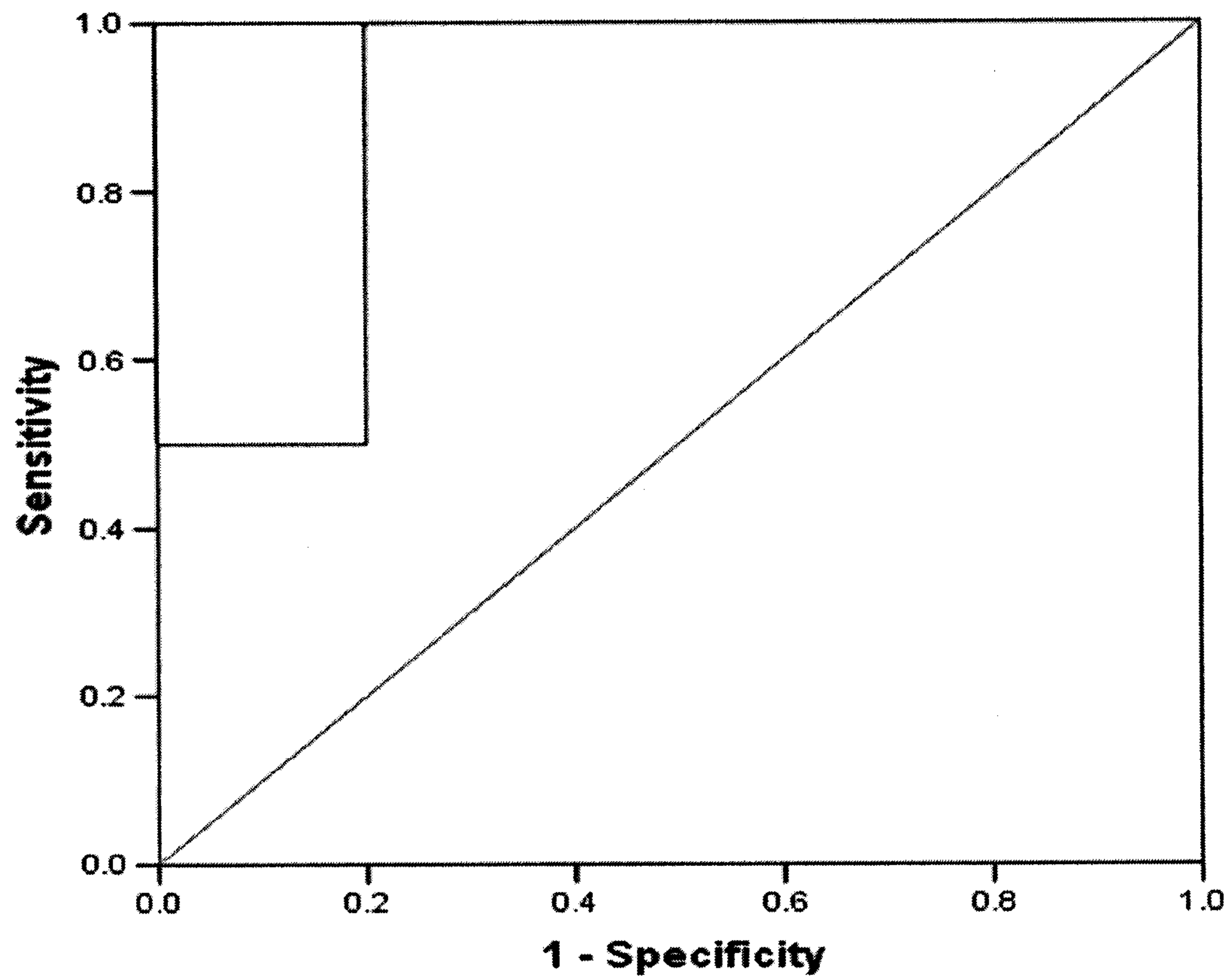
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
1.000	.000	.007	1.000	1.000

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 13b (cond.)

96/133

Non-Seminoma to Seminoma**ROC Curve****Figure 13b (cond.)**

97/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-1.7974	.000	.000
-.6304	.250	.000
-.3218	.500	.000
.0290	.500	.200
.2892	.750	.200
.9884	1.000	.200
2.1680	1.000	.400
2.7276	1.000	.600
3.0782	1.000	.800
4.4005	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.900	.112	.050	.681	1.119

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 13b (cond.)

Transcript 12

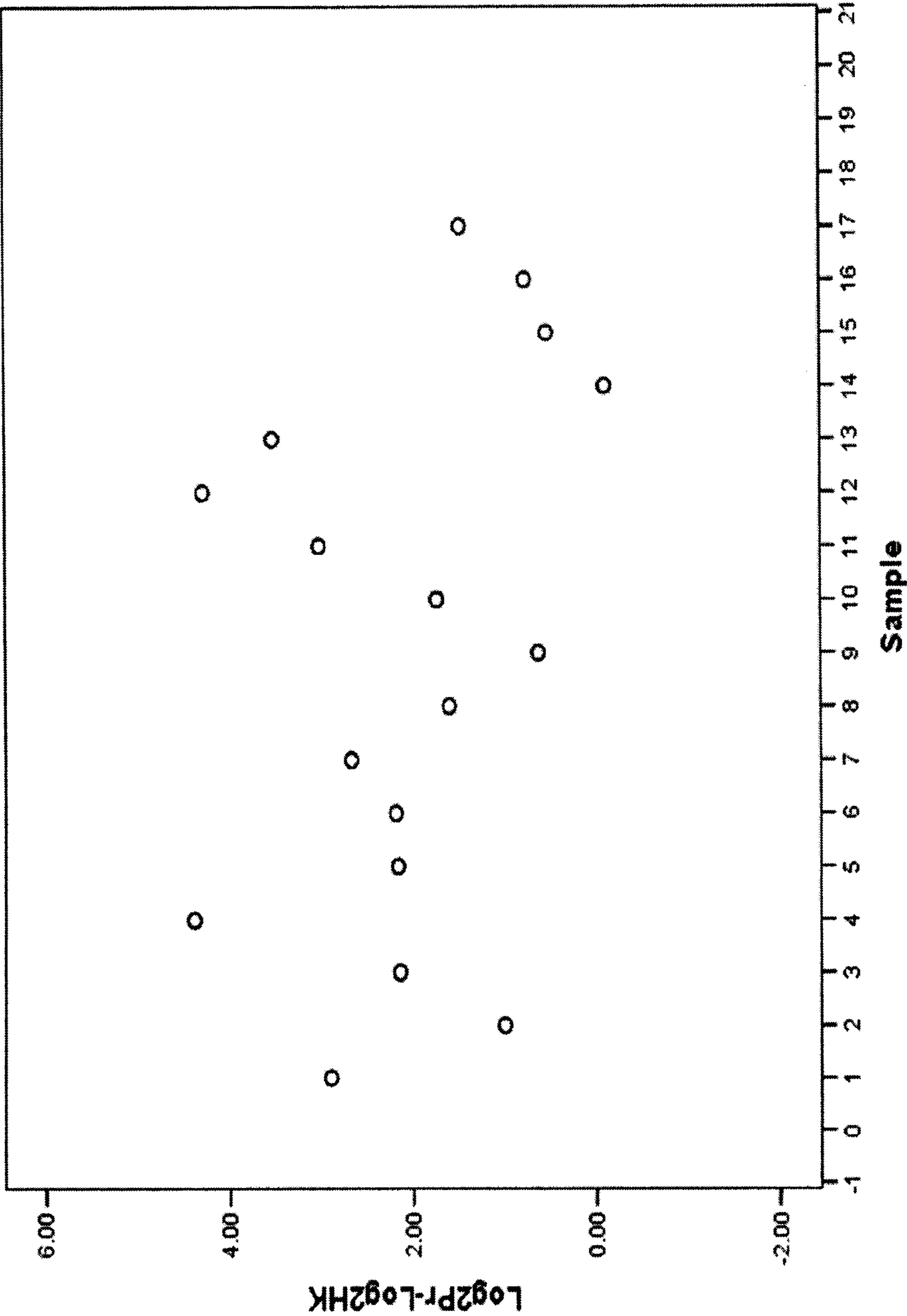


Figure 14a

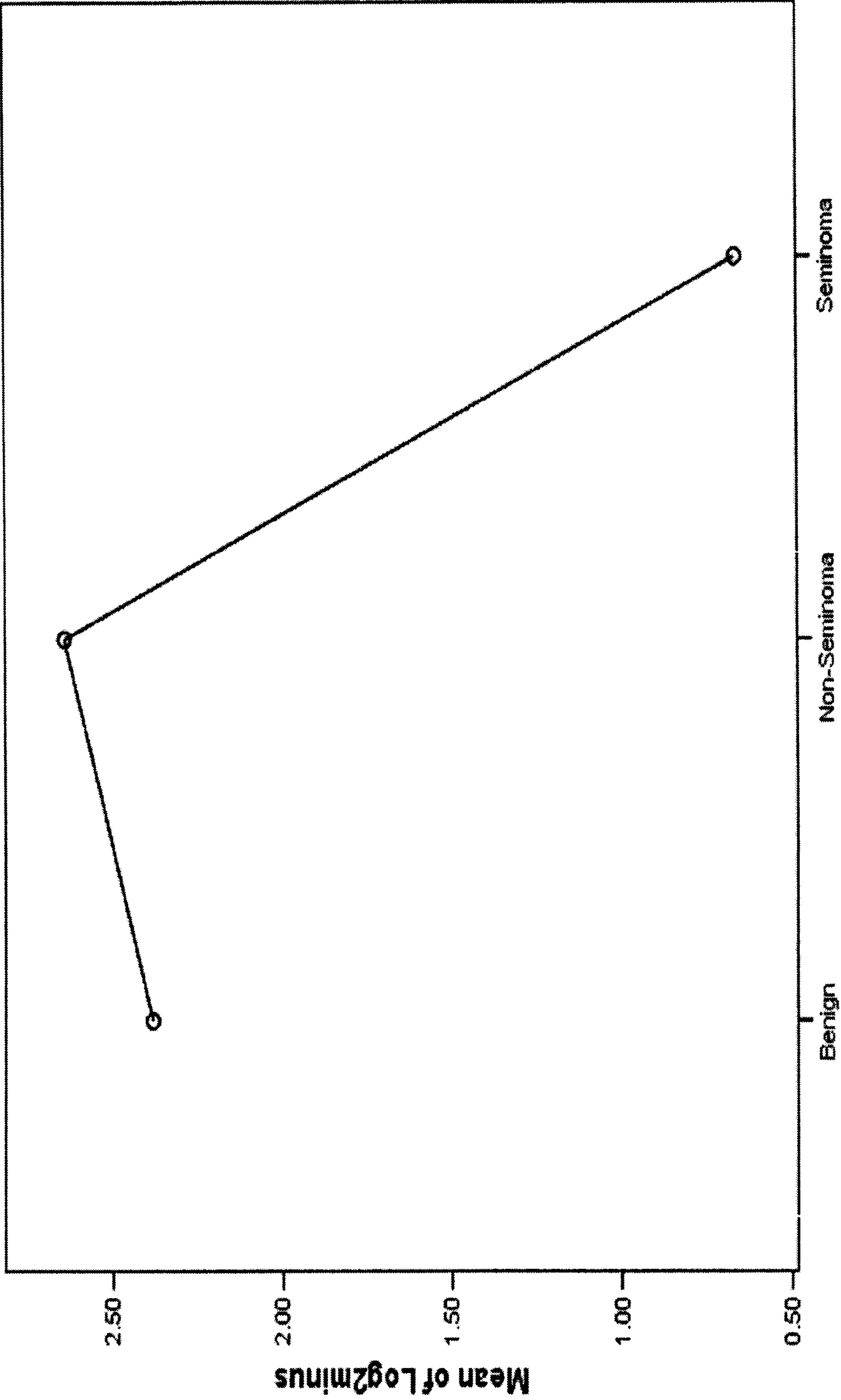
Multiple Comparisons

Dependent Variable: Log2Pr-Log2HK
Tukey HSD

(I) Secondary Diagnosis	(J) Secondary Diagnosis	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Benign	Non-Seminoma	-.25989	.62543	.910	-1.8968	1.3770
	Seminoma	1.71757	.67182	.056	-.0408	3.4759
Non-Seminoma	Benign	.25989	.62543	.910	-1.3770	1.8968
	Seminoma	1.97745*	.73594	.044	.0513	3.9036
Seminoma	Benign	-1.71757	.67182	.056	-3.4759	.0408
	Non-Seminoma	-1.97745*	.73594	.044	-3.9036	-.0513

*. The mean difference is significant at the .05 level.

Figure 14a (cond.)



Secondary Diagnosis

Figure 14a (cond.)

101/133

Benign to Seminoma

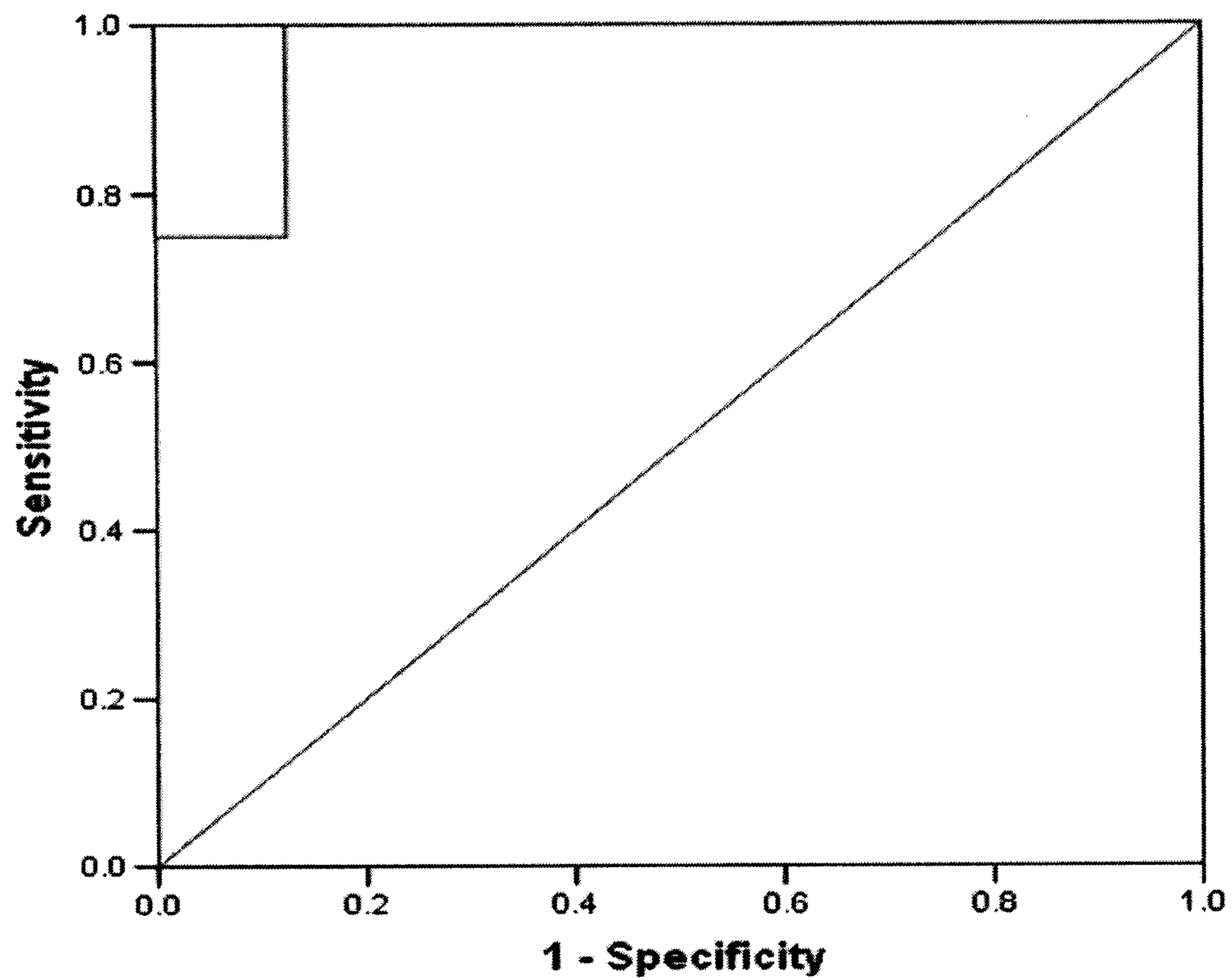
ROC Curve

Figure 14b

102/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-1.1036	.000	.000
.2116	.250	.000
.6444	.500	.000
.8799	.750	.000
1.2345	.750	.125
1.5361	1.000	.125
1.8719	1.000	.250
2.1521	1.000	.375
2.1746	1.000	.500
2.4309	1.000	.625
2.7874	1.000	.750
3.6445	1.000	.875
5.3880	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

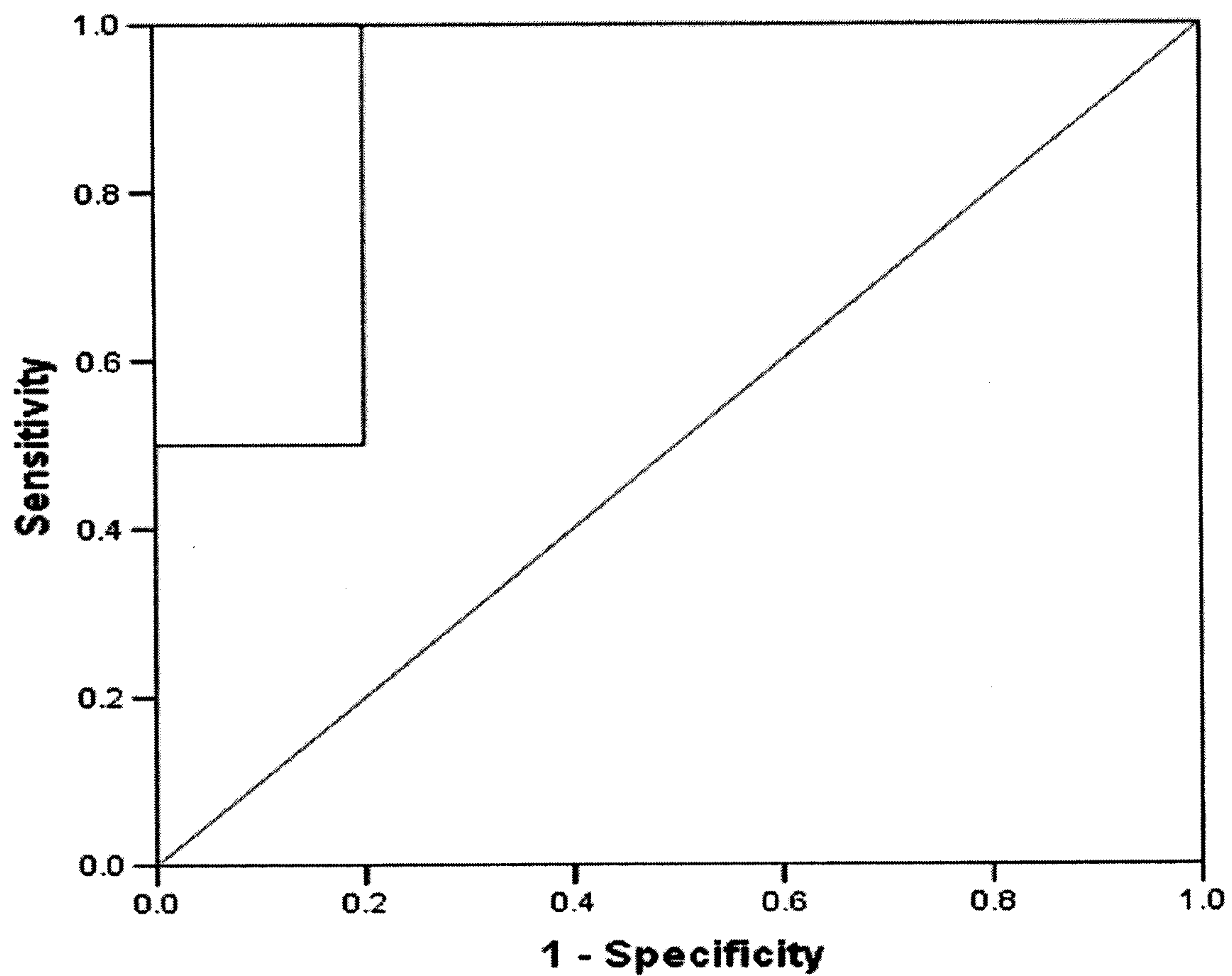
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.969	.047	.011	.876	1.061

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 14b (cond.)

103/133

Non-Seminoma to Seminoma**ROC Curve****Figure 14b (cond.)**

104/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-1.1036	.000	.000
.2116	.250	.000
.5766	.500	.000
.6942	.500	.200
1.1167	.750	.200
1.6039	1.000	.200
2.3812	1.000	.400
3.2778	1.000	.600
3.9096	1.000	.800
5.2895	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.900	.112	.050	.681	1.119

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 14b (cond.)

Transcript 13
Benign to Malignant

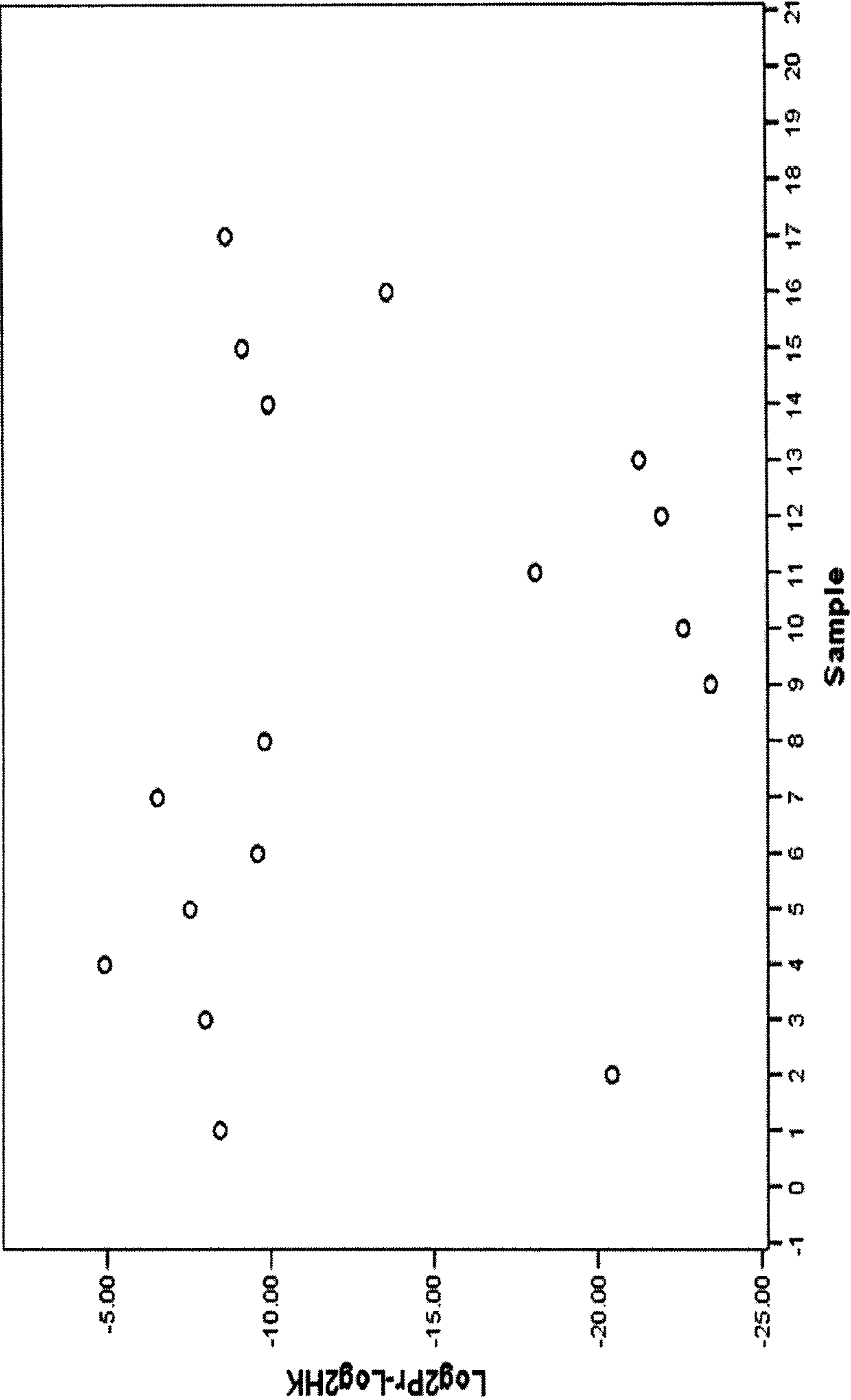


Figure 15a

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Log2Pr-Log2HK	Equal variances assumed	3.445	.083	2.638	15	.019	7.12469	2.70079	1.36810	12.88129
	Equal variances not assumed			2.682	14.703	.017	7.12469	2.65646	1.45261	12.79678

Figure 15a (cond.)

107/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-24.4381	1.000	1.000
-23.0168	1.000	.889
-22.2756	1.000	.778
-21.6134	1.000	.667
-20.8467	1.000	.556
-19.2627	.875	.556
-15.8501	.875	.444
-11.7732	.875	.333
-9.8751	.875	.222
-9.6932	.750	.222
-9.3712	.625	.222
-8.9122	.625	.111
-8.5527	.625	.000
-8.2104	.500	.000
-7.7549	.375	.000
-7.0316	.250	.000
-5.7275	.125	.000
-3.9187	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

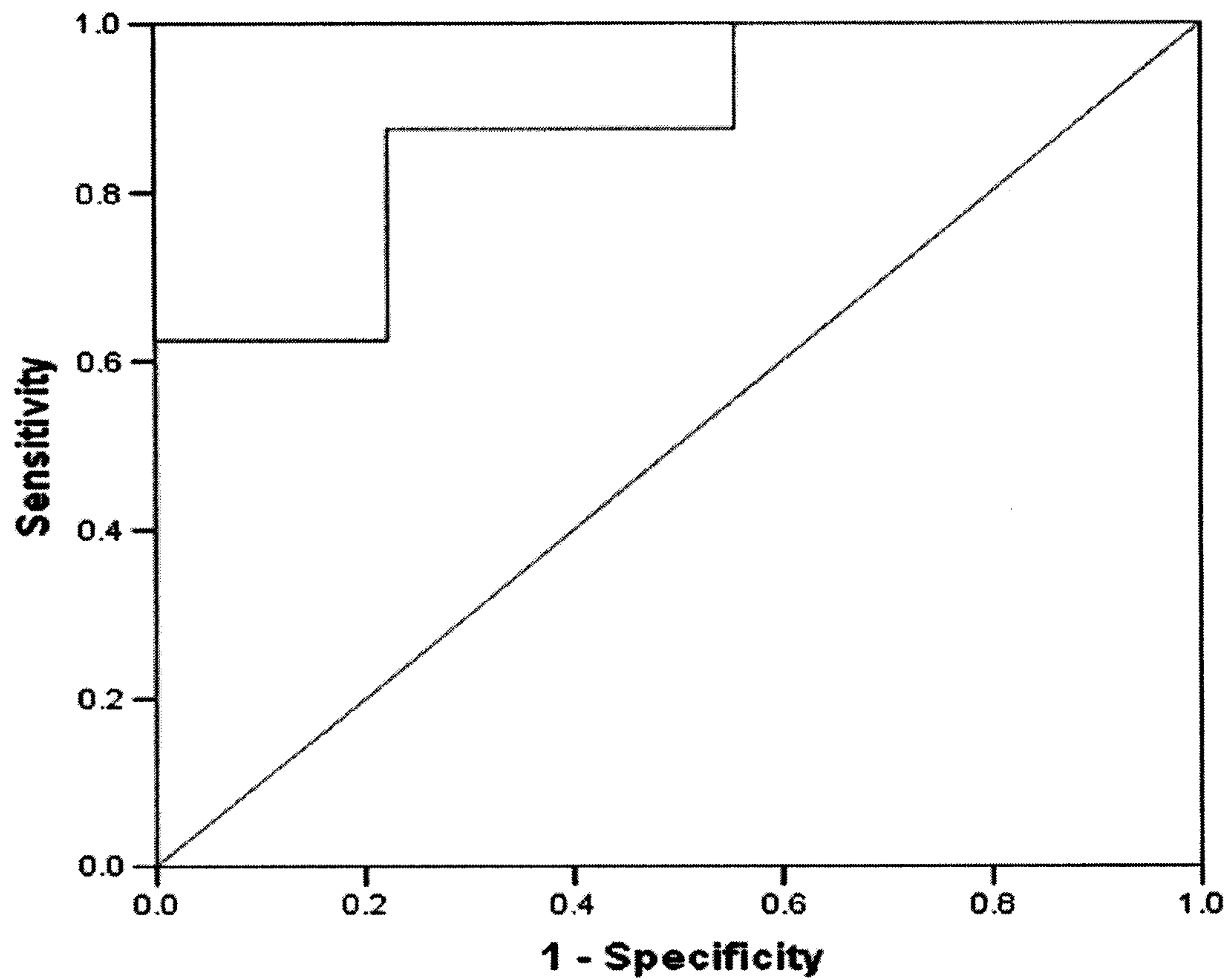
Test Result Variable(s): Log2Pr-Log2HK

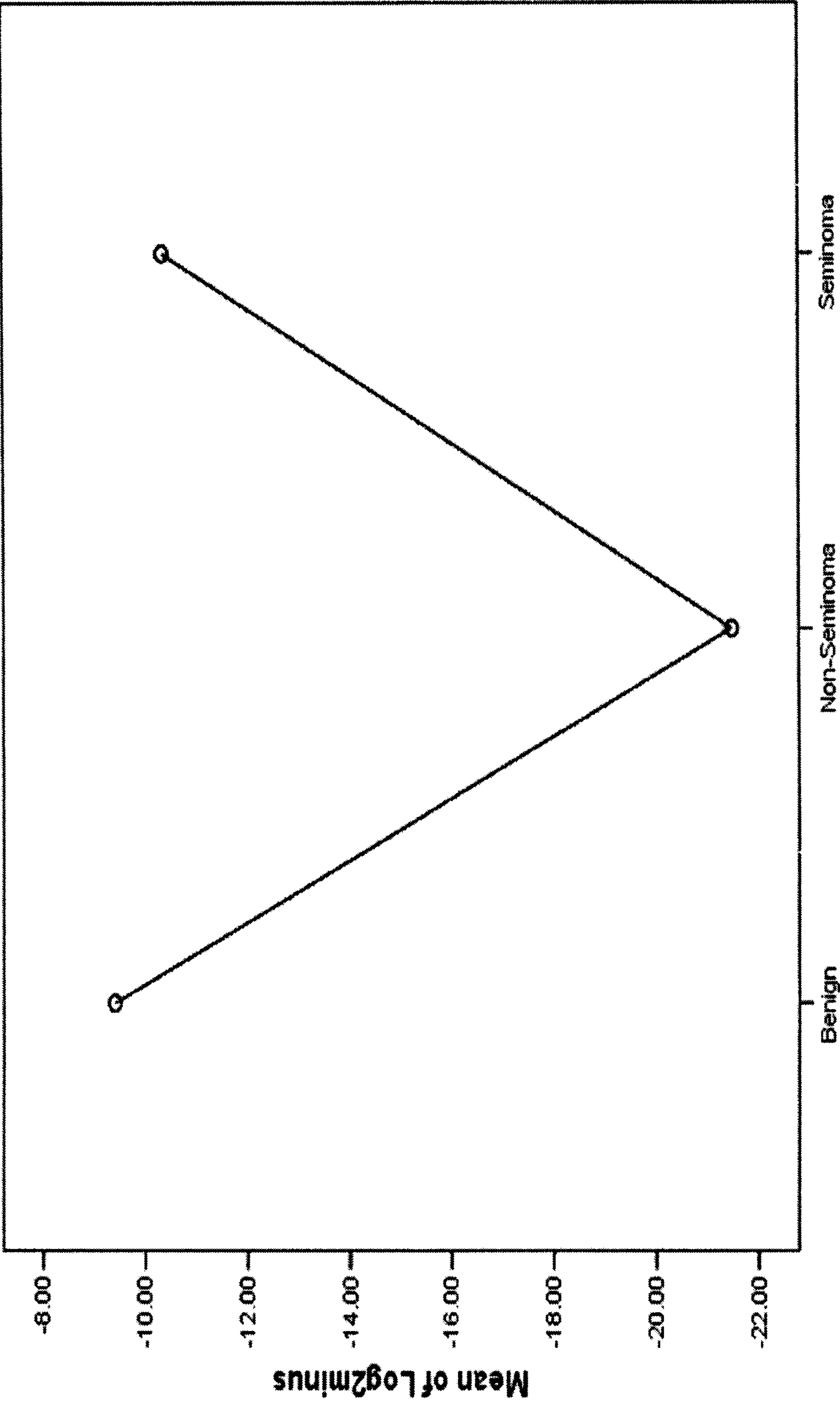
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.875	.086	.009	.706	1.044

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 15a (cond.)

108/133

ROC Curve**Figure 15a (cond.)**



Secondary Diagnosis

Figure 15a (cond.)

Multiple Comparisons

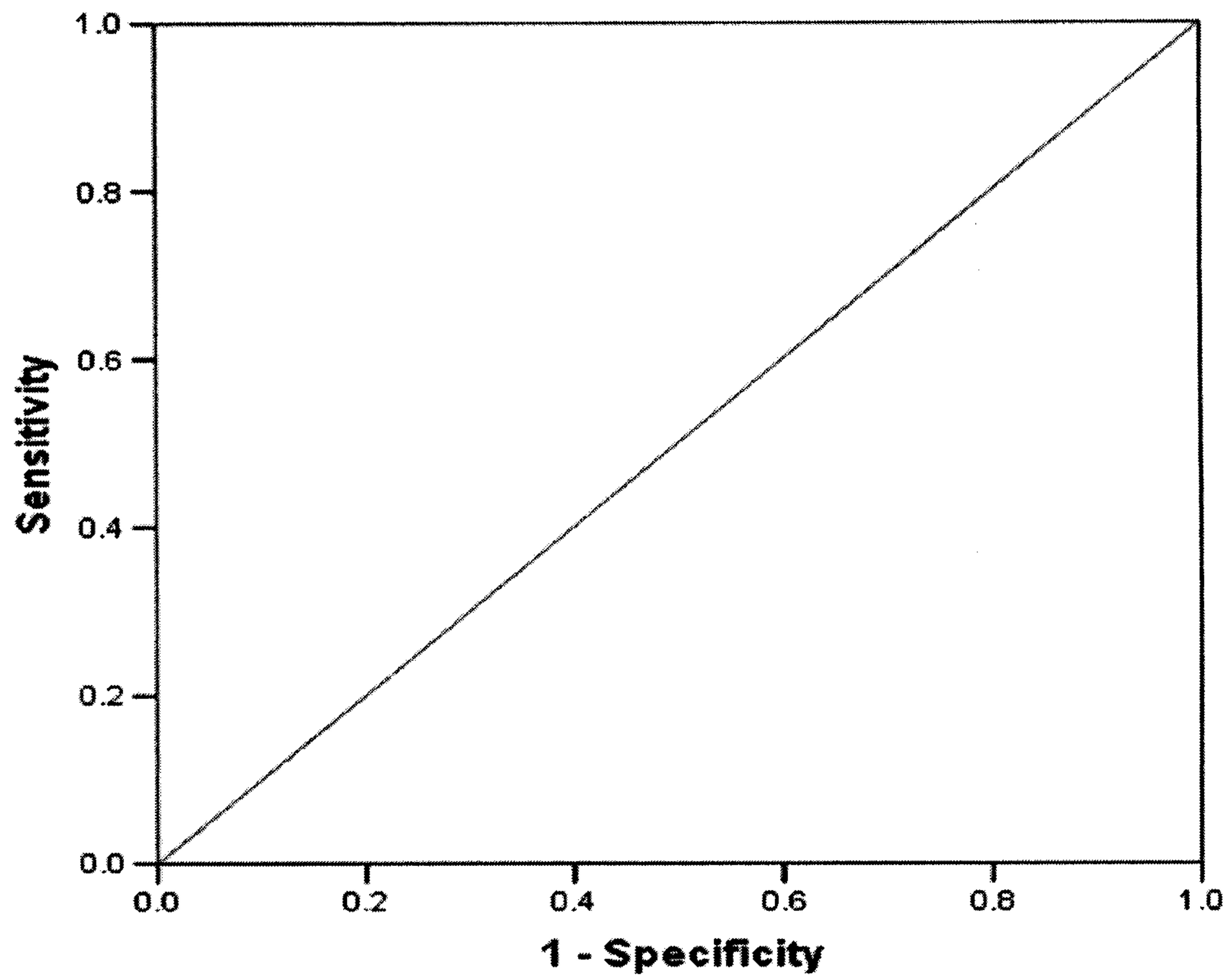
Dependent Variable: Log2Pr-Log2HK
Tukey HSD

(I) Secondary Diagnosis	(J) Secondary Diagnosis	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Benign	Non-Seminoma	12.07137*	2.08974	.000	6.6019	17.5408
	Seminoma	.94135	2.24474	.908	-4.9338	6.8165
Non-Seminoma	Benign	-12.07137*	2.08974	.000	-17.5408	-6.6019
	Seminoma	-11.13002*	2.45899	.001	-17.5659	-4.6941
Seminoma	Benign	-.94135	2.24474	.908	-6.8165	4.9338
	Non-Seminoma	11.13002*	2.45899	.001	4.6941	17.5659

*. The mean difference is significant at the .05 level.

Figure 15a (cond.)

111/133

Non-Seminoma to Seminoma**ROC Curve****Figure 15b**

112/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-24.4381	1.000	1.000
-23.0168	1.000	.800
-22.2756	1.000	.600
-21.6134	1.000	.400
-19.6872	1.000	.200
-15.8501	1.000	.000
-11.7732	.750	.000
-9.5531	.500	.000
-8.9122	.250	.000
-7.6678	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

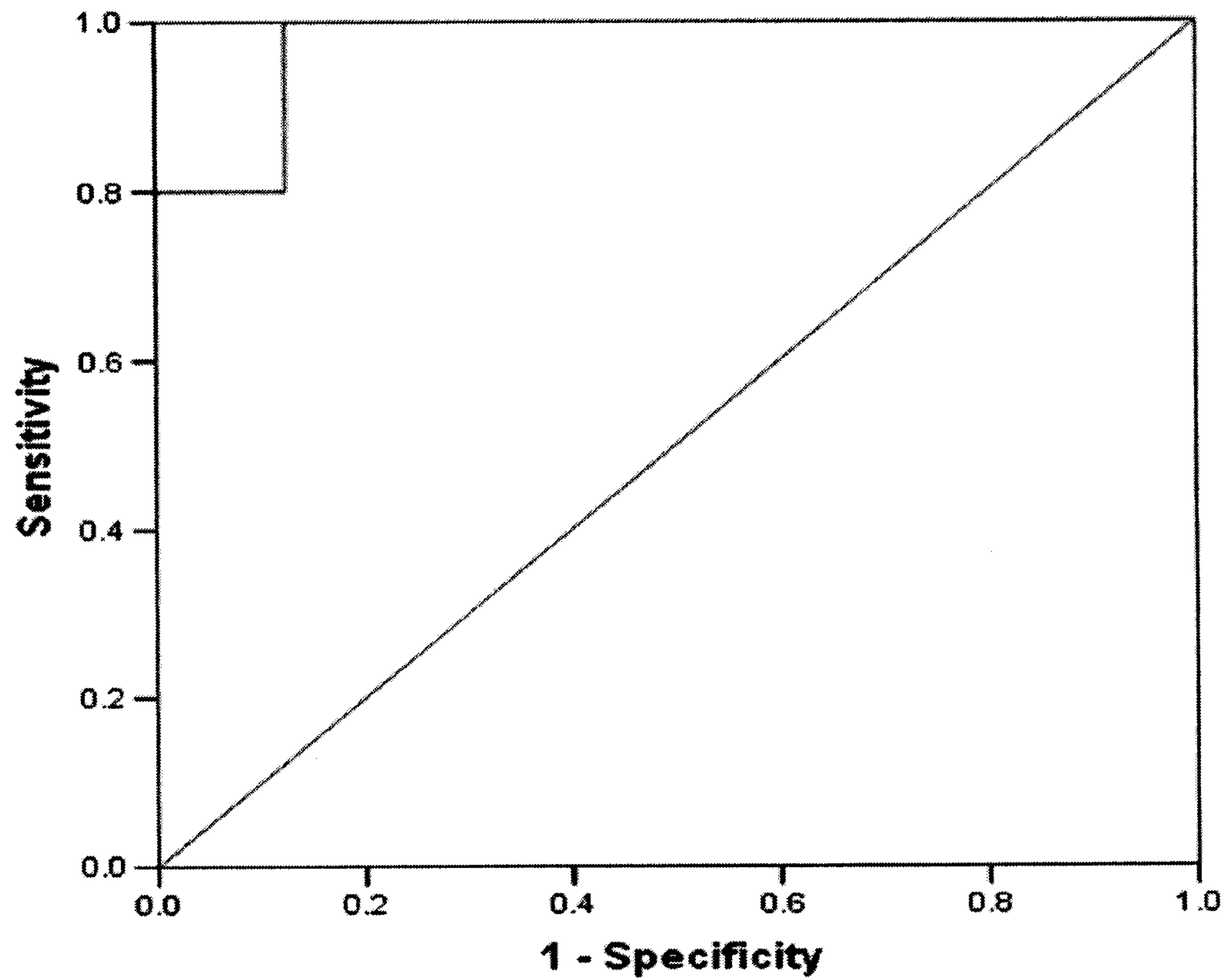
Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95 % Confidence Interval	
			Lower Bound	Upper Bound
1.000	.000	.014	1.000	1.000

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 15b (cond.)

113/133

Benign to Non-Seminoma**ROC Curve****Figure 15b (cond.)**

114/133

Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-24.4381	.000	.000
-23.0168	.200	.000
-22.2756	.400	.000
-21.6134	.600	.000
-20.8467	.800	.000
-19.2627	.800	.125
-13.9519	1.000	.125
-9.6932	1.000	.250
-9.0117	1.000	.375
-8.2104	1.000	.500
-7.7549	1.000	.625
-7.0316	1.000	.750
-5.7275	1.000	.875
-3.9187	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Case Processing Summary

Diagnosis	Valid N (listwise)
Positive ^a	5
Negative	8
Missing	4

Smaller values of the test result variable (s) indicate stronger evidence for a positive actual state.

- a. The positive actual state is Seminoma.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.975	.039	.005	.899	1.051

- a. Under the nonparametric assumption

- b. Null hypothesis: true area = 0.5

Figure 15b (cond.)

Transcript 15
Benign to Malignant

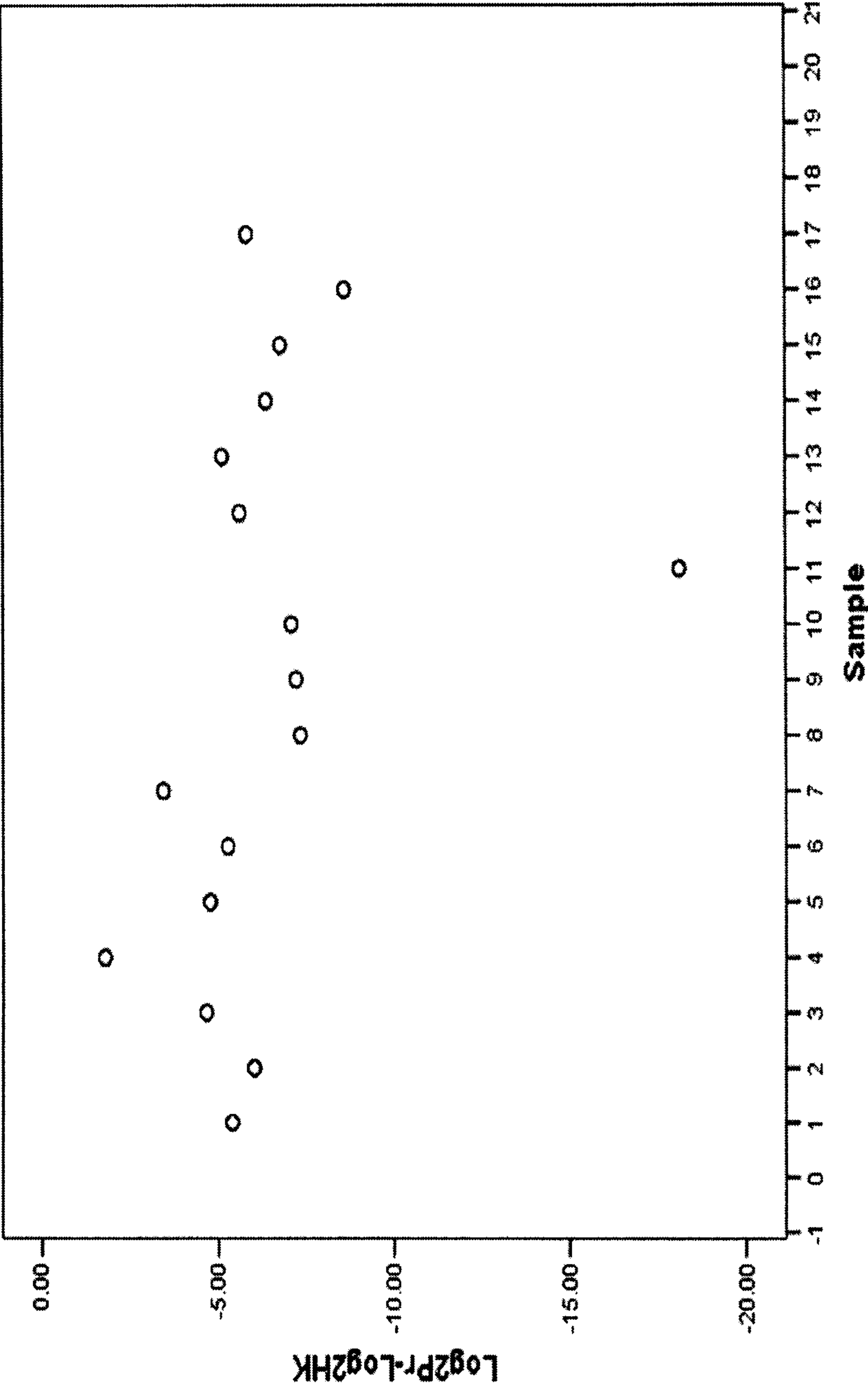
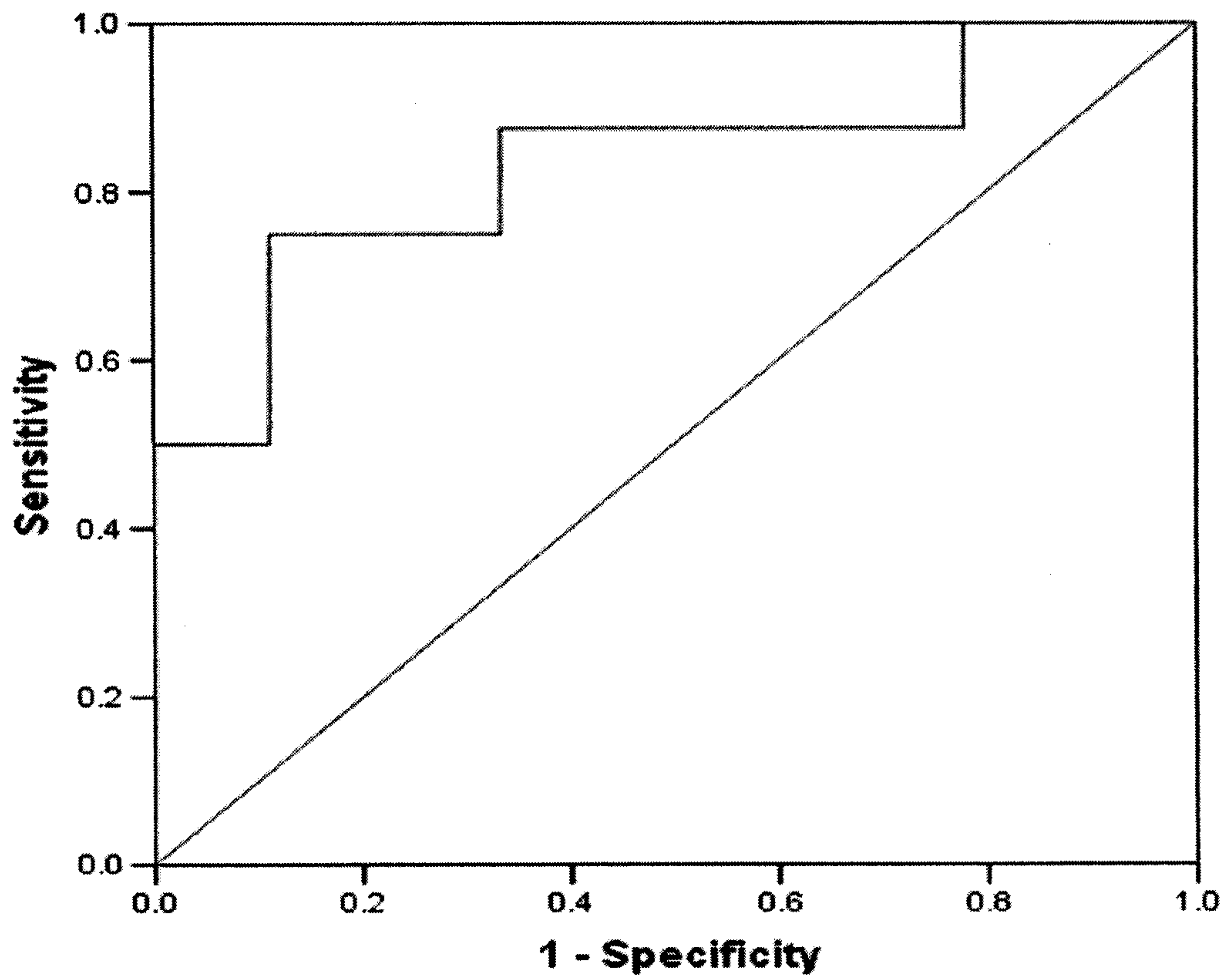


Figure 16

Independent Samples Test									
	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Log2Pr-Log2HK	1.272	.277	1.995	15	.065	3.02635	1.51727	-20763	6.26033
			2.084	10.974	.061	3.02635	1.45201	-.17044	6.22313

Figure 16 (cond.)

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ROC Curve**Figure 16 (cond.)**

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Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Greater Than or Equal To ^a	Sensitivity	1 - Specificity
-19.1032	1.000	1.000
-13.3553	1.000	.889
-7.9658	1.000	.778
-7.2682	.875	.778
-7.1398	.875	.667
-6.9179	.875	.556
-6.5654	.875	.444
-6.1847	.875	.333
-5.9128	.750	.333
-5.7090	.750	.222
-5.4916	.750	.111
-5.3213	.625	.111
-5.1819	.500	.111
-4.9299	.500	.000
-4.7011	.375	.000
-4.0399	.250	.000
-2.6039	.125	.000
-.7752	.000	.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.833	.106	.021	.626	1.040

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 16 (cond.)

Transcript 16
Benign to Malignant

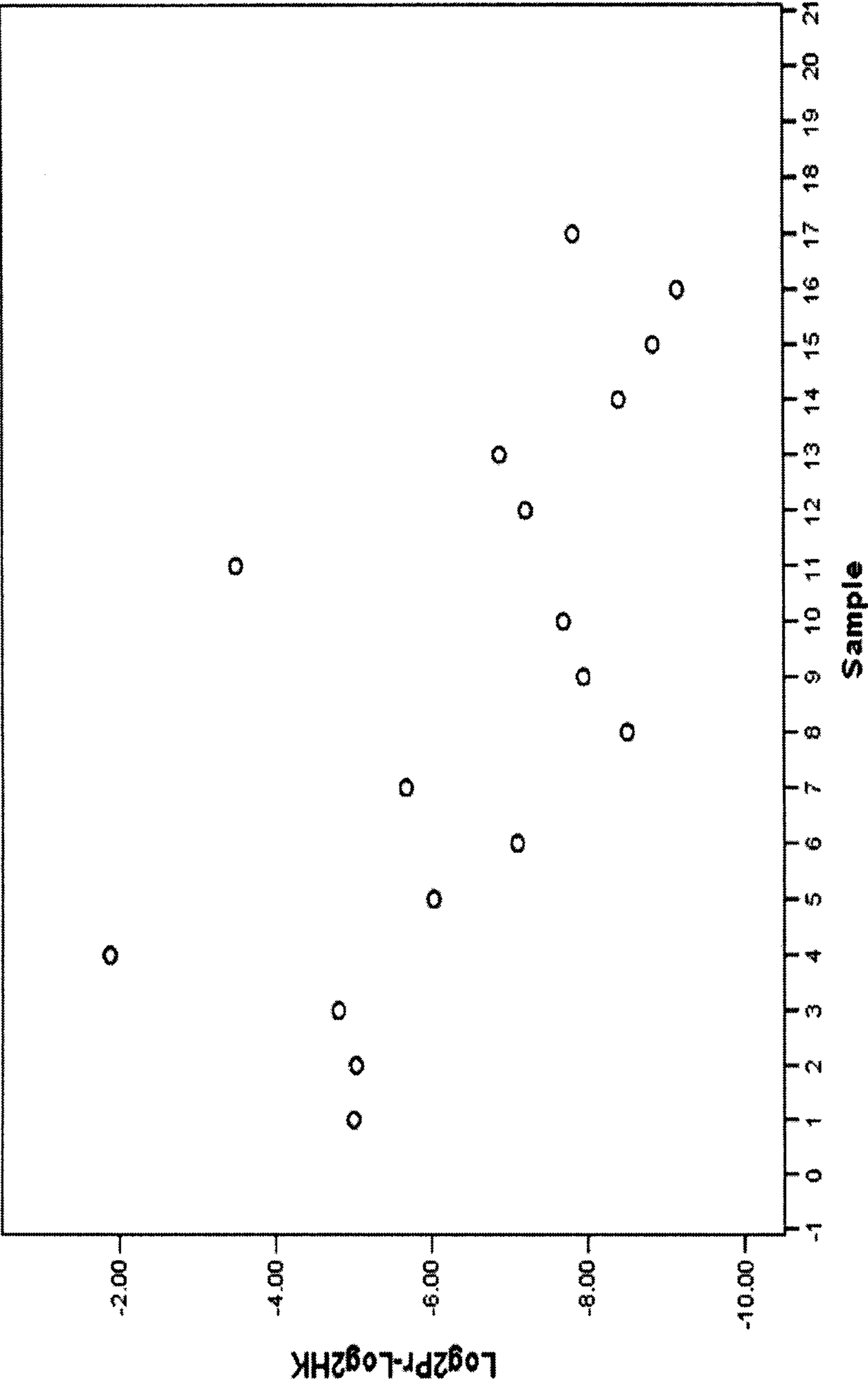
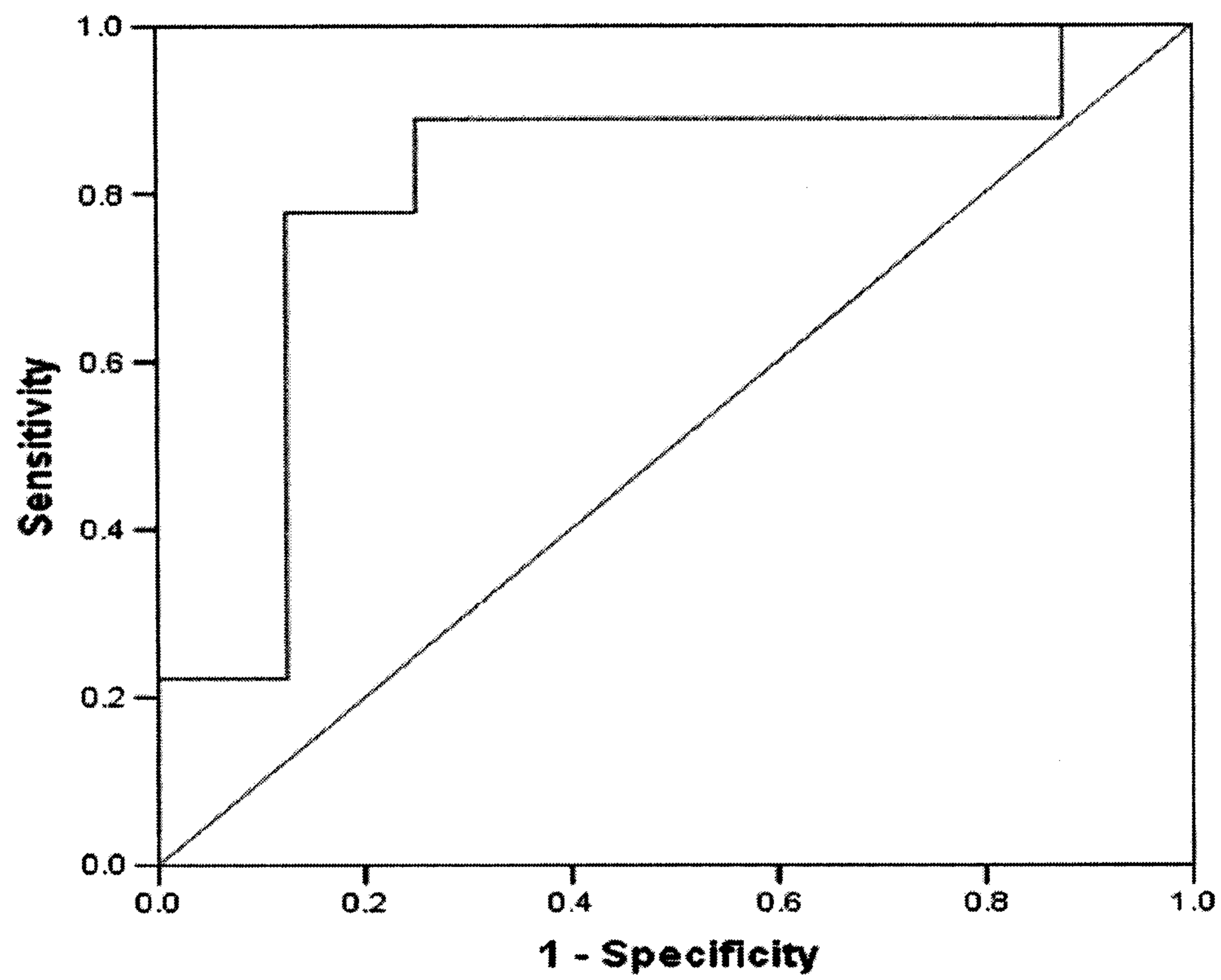


Figure 17a

Independent Samples Test										
	Levene's Test for Equality of Variances		t-test for Equality of Means							
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
								Lower	Upper	
Log2Pr-Log2HK	.149	.705	2.285	15	.037	1.98991	.87082	.13381	3.84602	
			2.265	13.995	.040	1.98991	.87870	.10522	3.87461	

Figure 17a (cond.)

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ROC Curve**Figure 17a (cond.)**

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Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-10.1467	.000	.000
-8.9916	.111	.000
-8.6680	.222	.000
-8.4482	.222	.125
-8.1689	.333	.125
-7.8794	.444	.125
-7.7537	.556	.125
-7.4470	.667	.125
-7.1508	.778	.125
-6.9861	.778	.250
-6.4480	.889	.250
-5.8459	.889	.375
-5.3490	.889	.500
-5.0125	.889	.625
-4.8989	.889	.750
-4.1432	.889	.875
-2.6805	1.000	.875
-.8743	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.806	.118	.034	.573	1.038

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 17a (cond.)

Multiple Comparisons

Dependent Variable: Log2Pr-Log2HK
Tukey HSD

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
(I) Secondary Diagnosis	(J) Secondary Diagnosis					
	Benign	1.14089	.96443	.482	-1.3833	3.6651
Non-Seminoma	Benign	-1.14089	.96443	.482	-3.6651	1.3833
	Seminoma	3.05119*	1.03596	.027	.3398	5.7626
Seminoma	Benign	-3.05119*	1.03596	.027	-5.7626	-.3398
	Non-Seminoma	-1.91029	1.13484	.246	-4.8805	1.0599

*. The mean difference is significant at the .05 level.

Figure 17a (cond.)

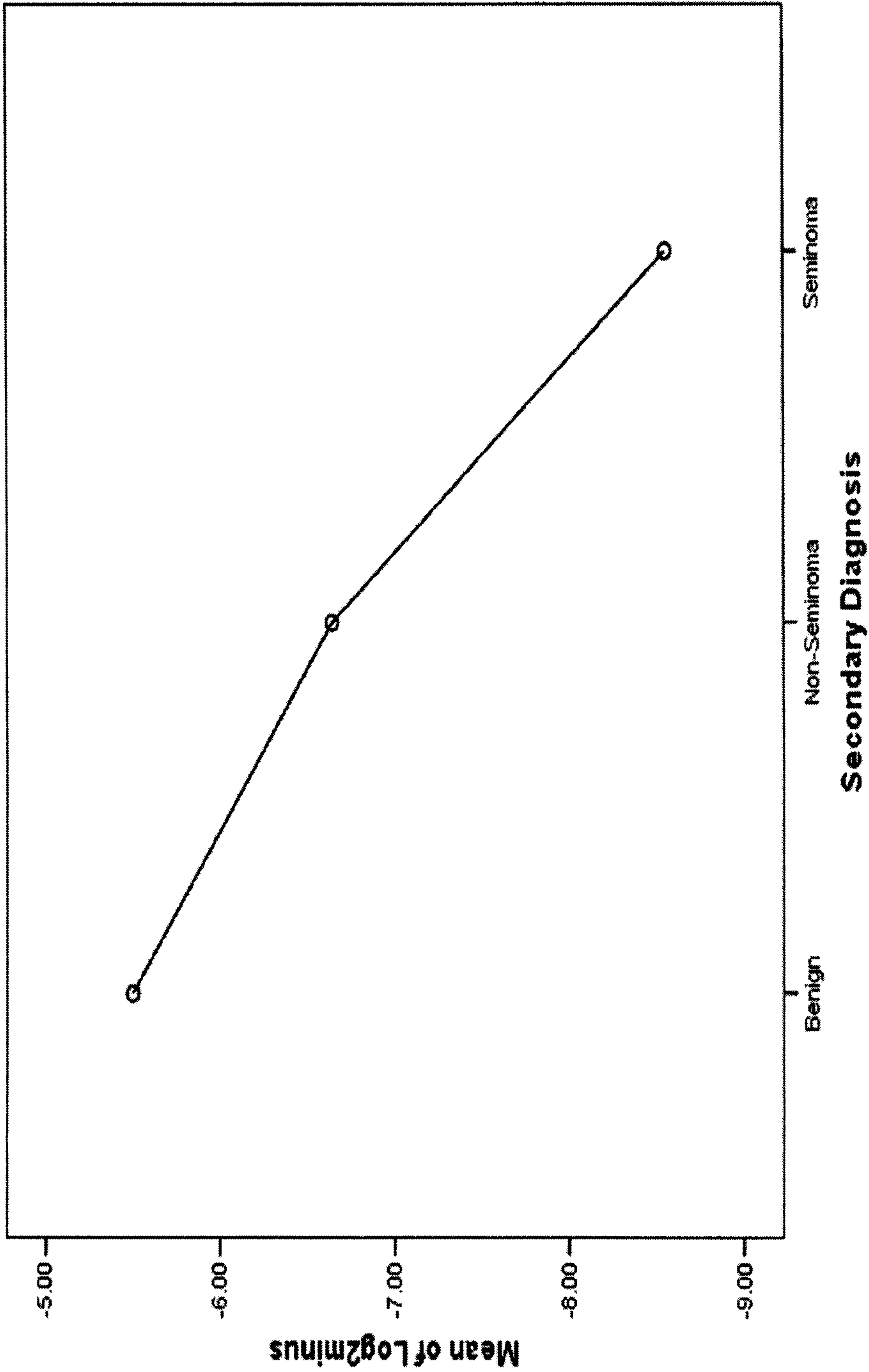
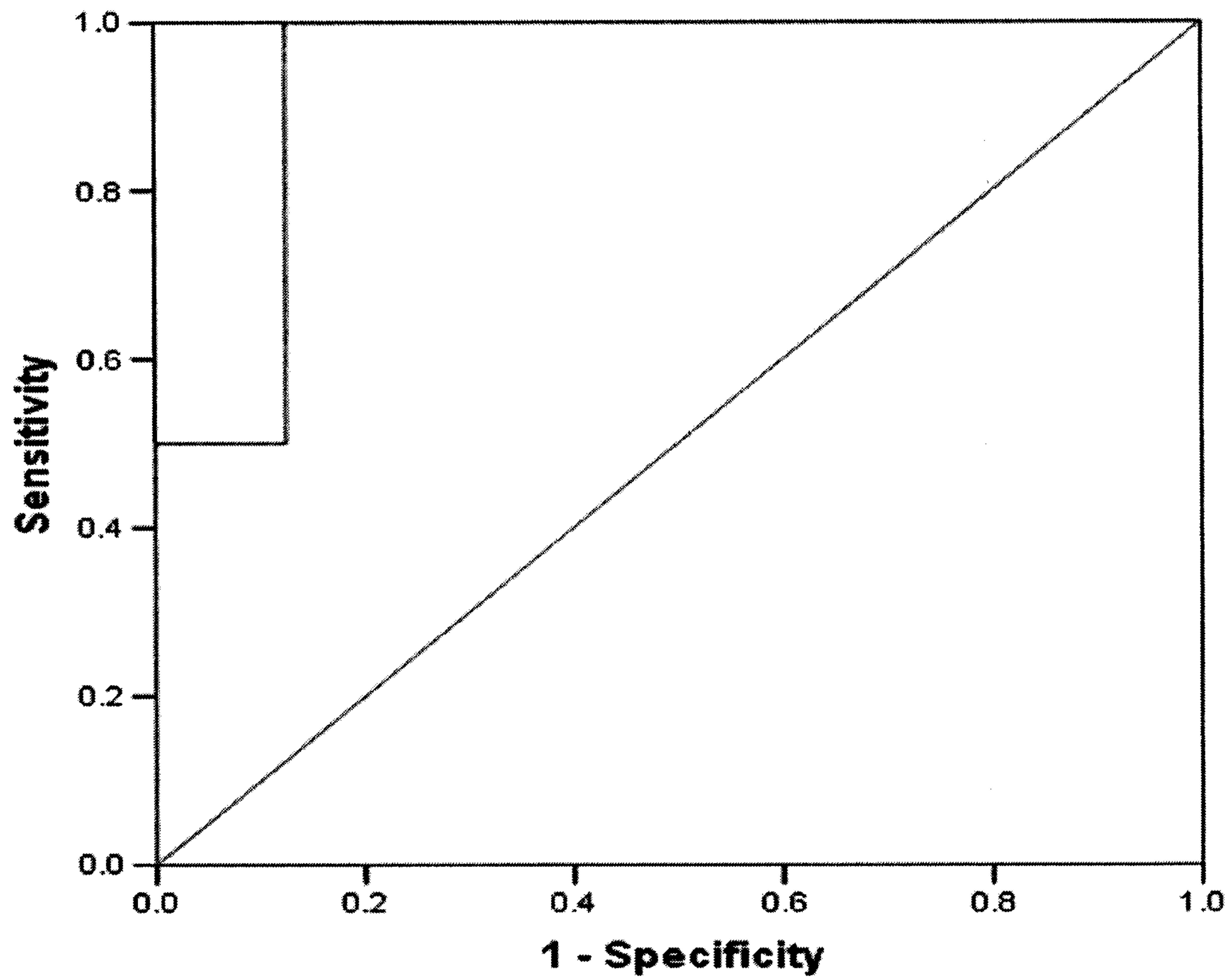


Figure 17a (cond.)

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Benign to Seminoma**ROC Curve****Figure 17b**

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Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-10.1467	.000	.000
-8.9916	.250	.000
-8.6680	.500	.000
-8.4482	.500	.125
-8.1075	.750	.125
-7.4575	1.000	.125
-6.5589	1.000	.250
-5.8459	1.000	.375
-5.3490	1.000	.500
-5.0125	1.000	.625
-4.8989	1.000	.750
-3.3371	1.000	.875
-.8743	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.938	.072	.017	.796	1.079

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 17b (cond.)

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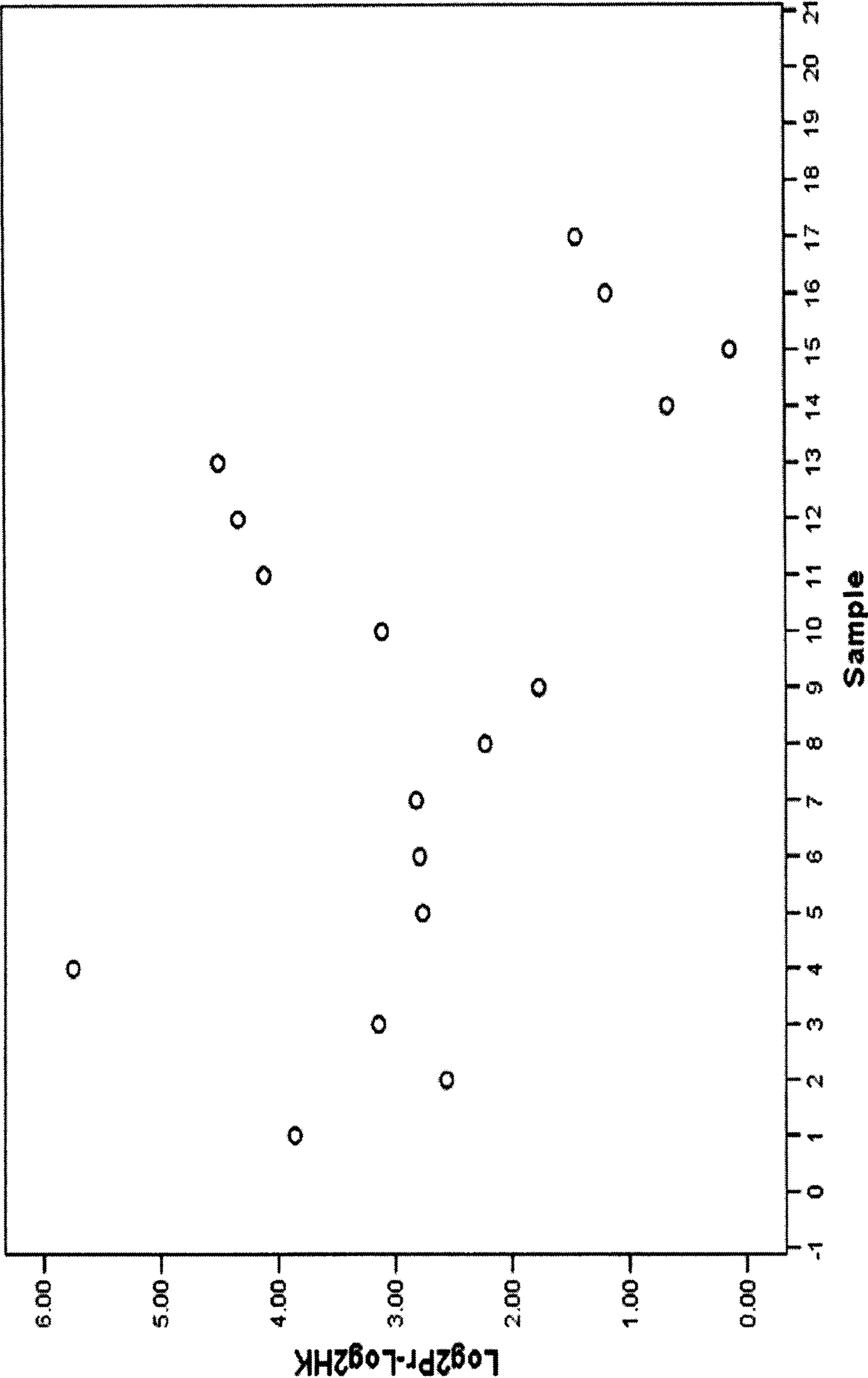


Figure 18a

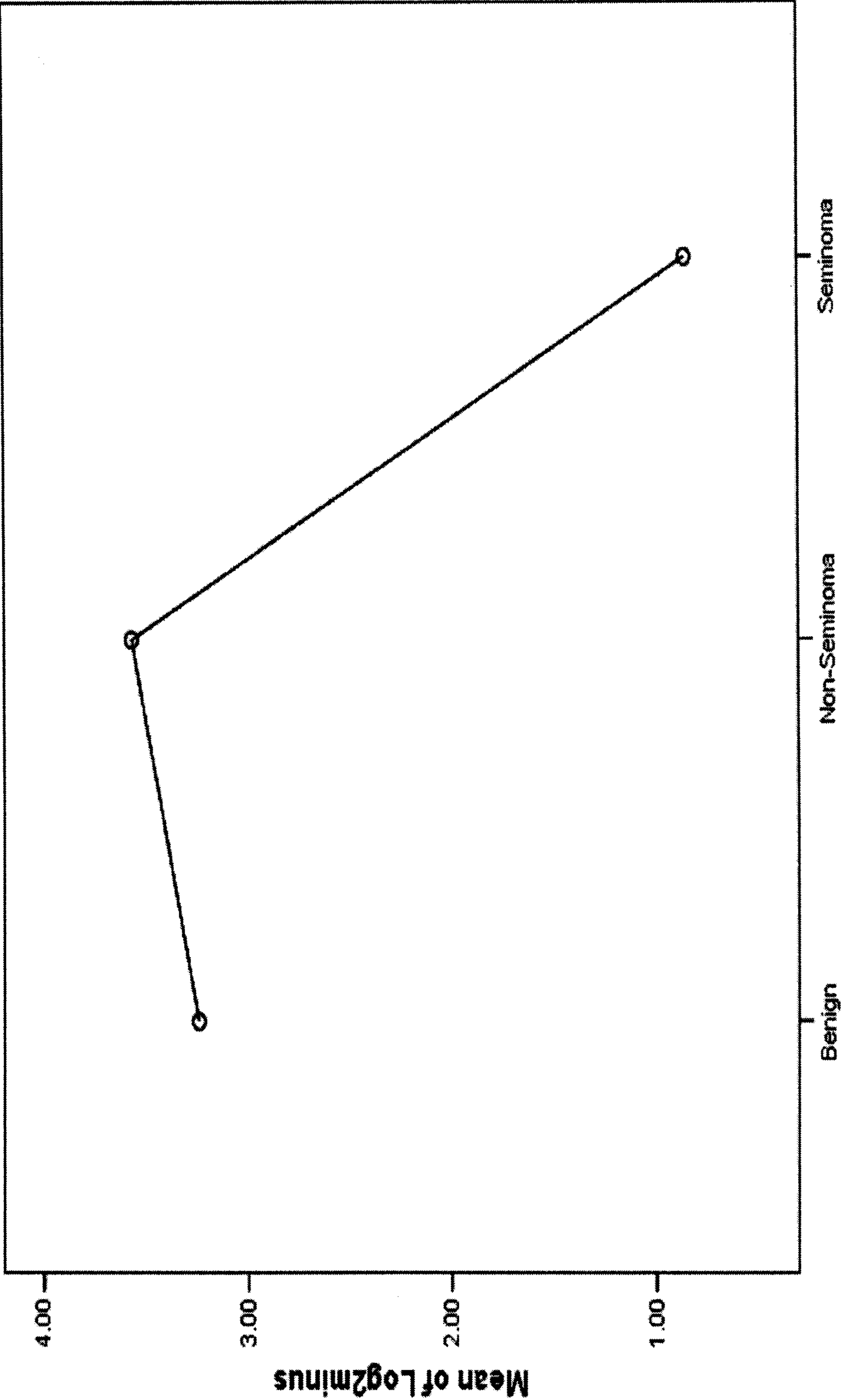
Multiple Comparisons

Dependent Variable: Log2Pr-Log2HK
Tukey HSD

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
(I) Secondary Diagnosis	(J) Secondary Diagnosis					
	Benign					
	Non-Seminoma	-.32805	.59050	.845	-1.8735	1.2174
	Seminoma	2.38275*	.63429	.006	.7226	4.0429
Non-Seminoma	Benign	.32805	.59050	.845	-1.2174	1.8735
	Seminoma	2.71080*	.69484	.004	.8922	4.5294
Seminoma	Benign	-2.38275*	.63429	.006	-4.0429	-.7226
	Non-Seminoma	-2.71080*	.69484	.004	-4.5294	-.8922

*. The mean difference is significant at the .05 level.

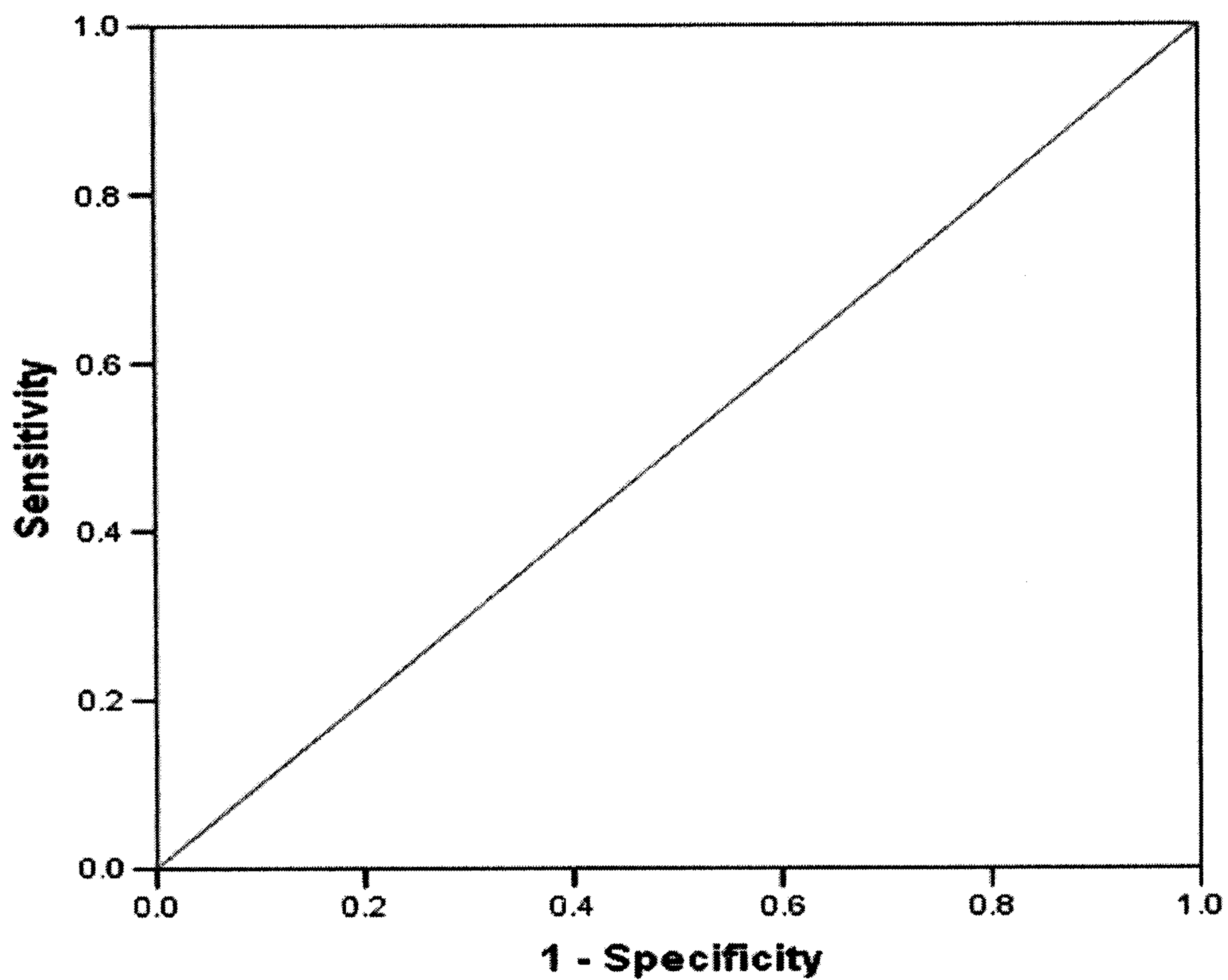
Figure 18a (cond.)



Secondary Diagnosis

Figure 18a (cond.)

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Benign to Seminoma**ROC Curve****Figure 18b**

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Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-.8670	.000	.000
.4000	.250	.000
.9279	.500	.000
1.3158	.750	.000
1.8364	1.000	.000
2.3956	1.000	.125
2.6632	1.000	.250
2.7784	1.000	.375
2.8058	1.000	.500
2.9821	1.000	.625
3.5019	1.000	.750
4.8065	1.000	.875
6.7531	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

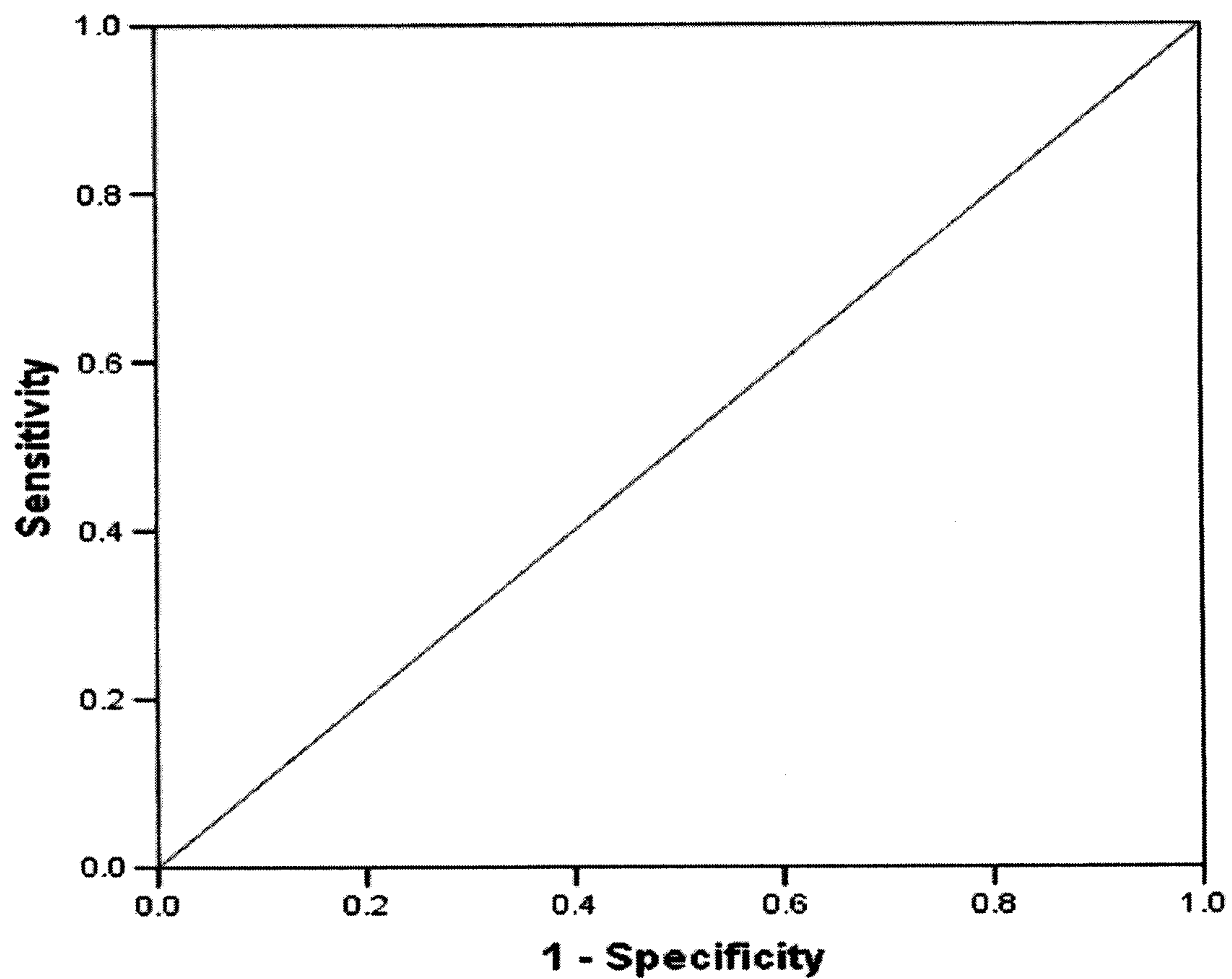
Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
1.000	.000	.007	1.000	1.000

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 18b (cond.)

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Non-Seminoma to Seminoma**ROC Curve****Figure 18b (cond.)**

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Coordinates of the Curve

Test Result Variable(s): Log2Pr-Log2HK

Positive if Less Than or Equal To ^a	Sensitivity	1 - Specificity
-.8670	.000	.000
.4000	.250	.000
.9279	.500	.000
1.3158	.750	.000
1.6065	1.000	.000
2.4411	1.000	.200
3.6145	1.000	.400
4.2275	1.000	.600
4.4220	1.000	.800
5.5063	1.000	1.000

- a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve

Test Result Variable(s): Log2Pr-Log2HK

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
1.000	.000	.014	1.000	1.000

- a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 18b (cond.)

ND4L

ND4

tRNA H, S, L

ND5



10745-10754

14124-14133