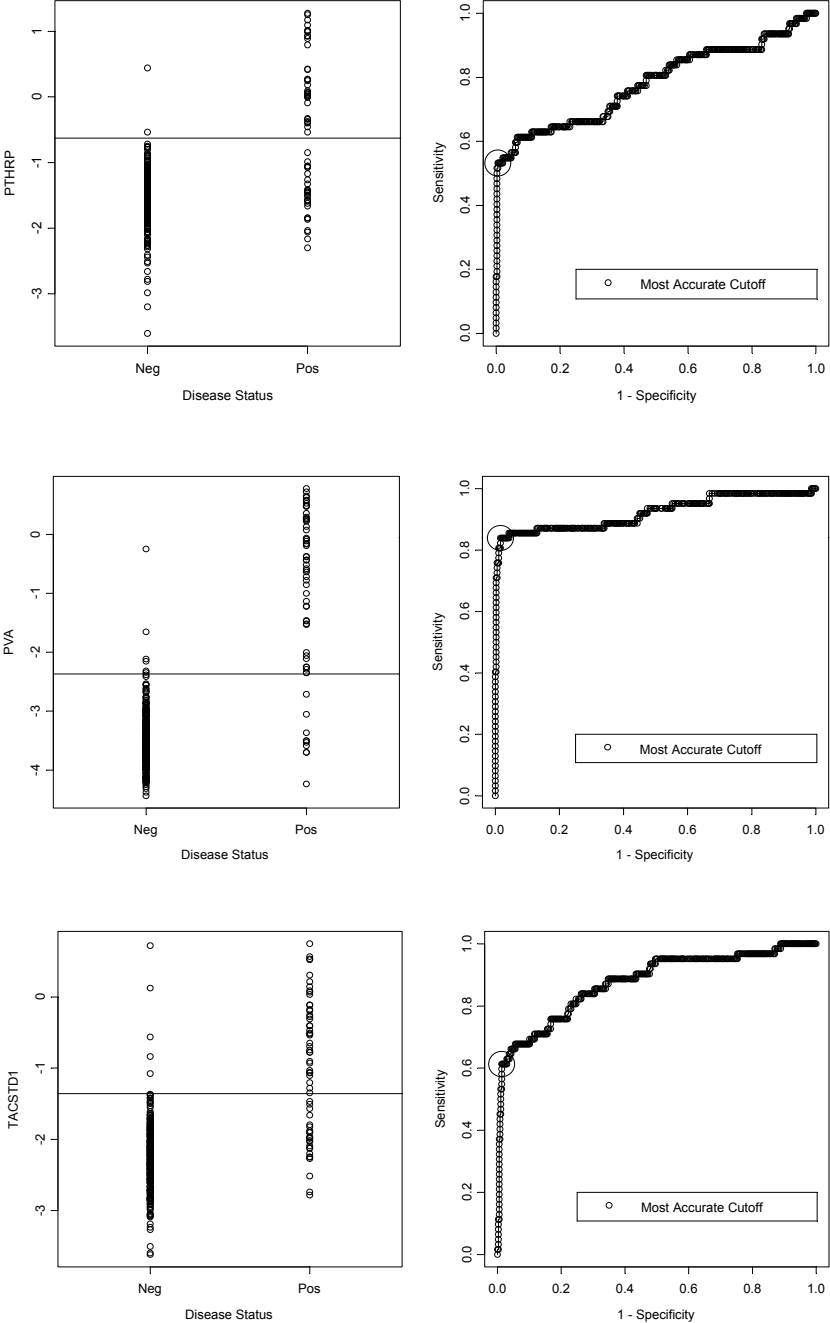
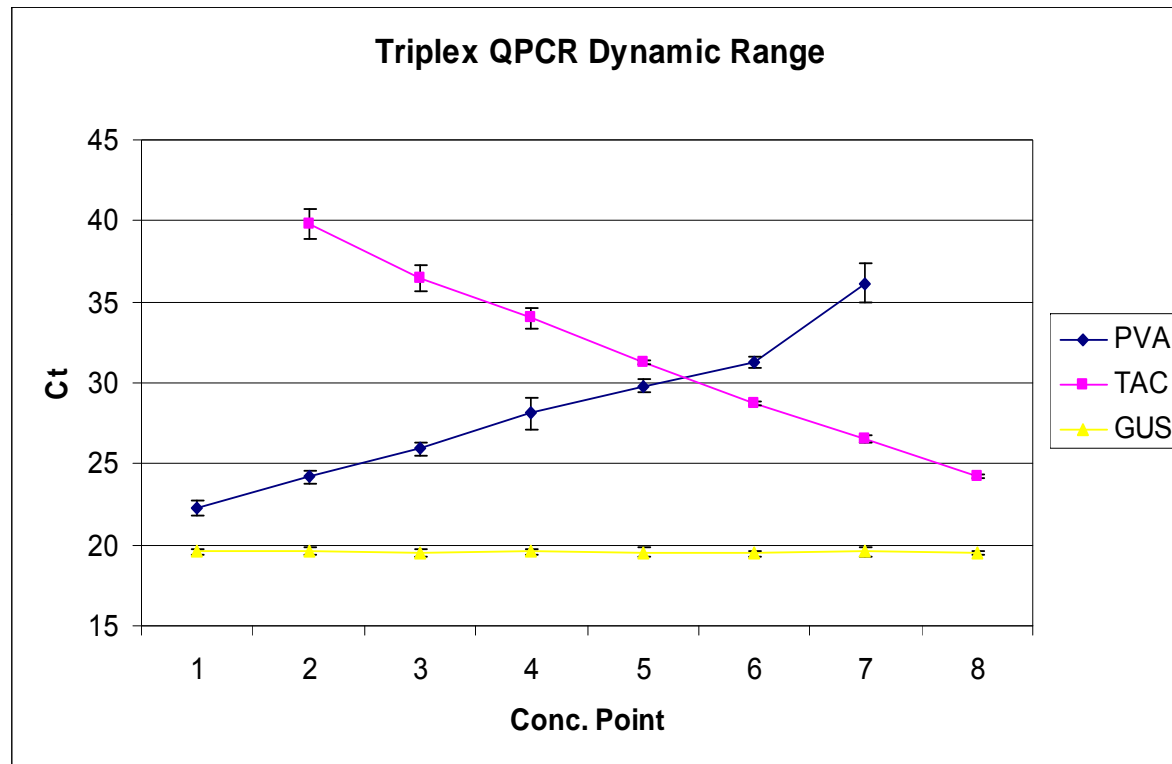


# Supplementary Fig S1

Distribution of relative expression (upper panel) of PTHRP, PVA and TACSTD1 in histologically positive and negative lymph nodes as determined by review of all tissue sections (consensus pathology). The Y-axis shows relative expression as delta CT and the horizontal line indicates the most accurate cut-off determined from ROC curve analysis (lower panel).

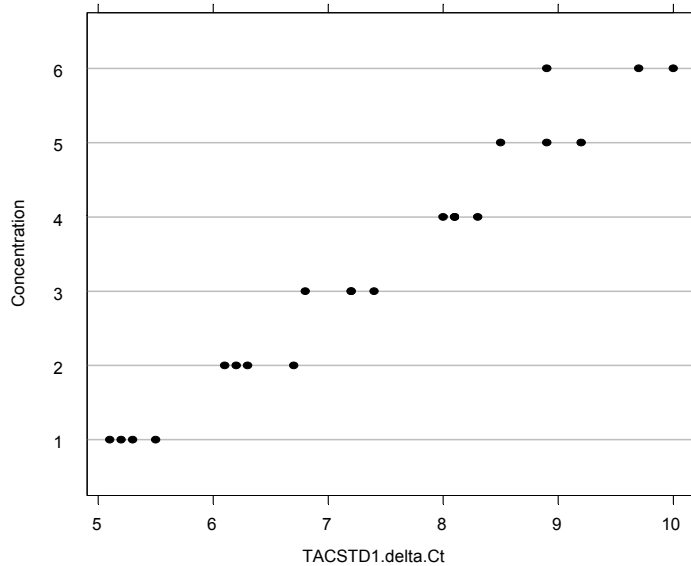


# Supplementary Figure S2

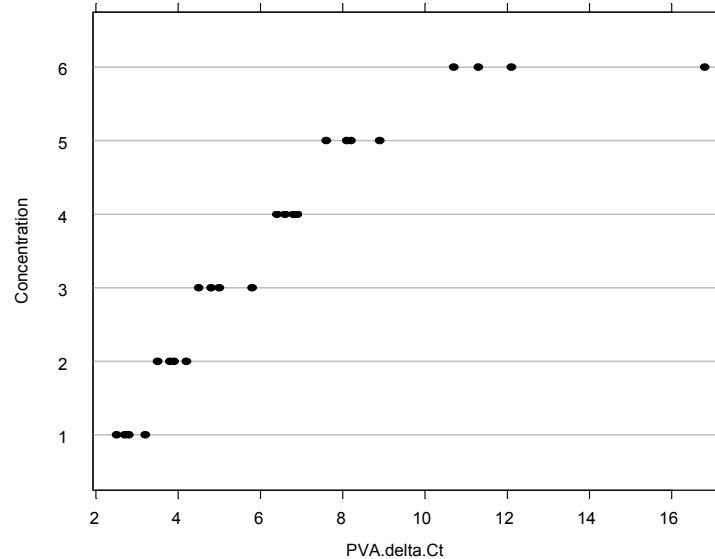


Dynamic range of markers PVA and TACSTD1 in the triplex assay.

# Supplementary Figure S3



Coefficient of Variation = 3.9%  
Intraclass Correlation Coefficient = .968  
Lower 95% Confidence Bound for ICC = .914  
Standard Error of Measurement = .297  
95% Prediction Interval for a New Measurement = .58 cycles



Coefficient of Variation = 18.2%  
Intraclass Correlation Coefficient = .898  
Lower 95% Confidence Bound for ICC = .751  
Standard Error of Measurement = 1.19  
95% Prediction Interval for a New Measurement = 2.34 cycles

## Supplementary Table S1. Oligonucleotide primer and probe sequences used in GeneXpert assay

Gene	Oligonucleotide	Sequence (5' → 3')
GUSB	RT primer	TTTGGT TGTCTCTGCCGAGT
	Forward primer	CTCATTGGGAATTTTGCCGATTCA
	Reverse primer	CCGAGTGAAGATCCCCTT
	Probe	TGACTGAACAGTCACCGACGAGAGTGCTGG
PVA	RT primer	ACAGGTCAACAGCAG
	Forward primer	* <b>GC</b> CTGTGGA ACTTCTTACCCAACCA
	Reverse primer	* <b>CA</b> AGGGGGGCCAACAGCA
	Probe	TGGCAGGCCGCACTCAGGGAGGC
TACSTD1	RT primer	AGTTTACGGCCAGCTTG
	Forward primer	* <b>GAC</b> GCGTTCGGGCTTCTGCTT
	Reverse primer	* <b>GCGA</b> AGTTTTTCACAGACACATTCTTCCTGAG
	Probe	CGGCGACGGCGACTTTTGC

\* Bases indicated in bold are non-complementary tails added to increase primer T<sub>m</sub> in later PCR cycles.

**Supplementary Table S2.** Summary table comparing clinical frozen section, research pathology and permanent pathology with overall consensus pathology as the gold standard. Details of each comparison are in Supplemental Tables B-D.

	<b>Sensitivity</b>	<b>Specificity*</b>	<b>Accuracy</b>
<b>Clinical Frozen Section</b>	80.3%	100%	97.2%
<b>Research Pathology</b>	86.9%	100%	98.2%
<b>Permanent Pathology</b>	91.8%	100%	98.9%

\* Specificity has to be 100% since overall pathology is a consensus of all three analyses.

## Supplementary Table S3. Research Pathology Final Call (H&E plus IHC): VS Overall Pathology

		Research			
		Pos	Neg	ITC	
Overall	Pos	53	8	1	Overall Accuracy 433/442 = 98%
	Neg	0	378	0	Overall Accuracy* 431/439 = 98.2%
	ITC	0	0	2	Sensitivity* 53/61 = 86.9%
					Specificity* 100%

\* Not including ITC)

448 nodes evaluated (6 with PTC removed). No non-diagnostic cases. N=442

# Supplementary Table S4. Clinical FS Pathology VS Overall Pathology

		Clinical FS		
		Pos	Neg	ITC
Overall	Pos	49	12	0
	Neg	0	374	0
	ITC	0	2	0
	Total	49	386	0

Overall Accuracy  
423/437 = 96.8%

Overall Accuracy\*  
423/435 = 97.2%

Sensitivity\*  
49/61 = 80.3%

Specificity\*  
100%

\* Not including ITC)

448 nodes evaluated (6 with PTC removed). 5 non-diagnostic on FS. N=437

## Supplementary Table S5. Clinical Permanent Pathology VS Overall Pathology

		Clinical Permanent			
		Pos	Neg	ITC	
Overall	Pos	56	5	2	Overall Accuracy 432/439 = 98.4%
	Neg	0	376	0	Overall Accuracy* 432/437 = 98.9%
	ITC	0	0	0	Sensitivity* 56/61 = 91.8%
					Specificity* 100%

\* Not including ITC)

448 nodes evaluated (6 with PTC removed). 3 non-diagnostic on perm. N=439