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Poster # 2085

Analytical Validation of a Serum miRNA RT-qPCR Assay for Monitoring Allograft Liver Rejection

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Introduction

- Liver function tests often indicate acute cellular rejection (ACR) in liver transplant (LTx) patients, but invasive liver biopsy remains the gold standard for definitive diagnosis.
- Existing efforts to develop minimally-invasive liquid biopsy assays have faced challenges, including suboptimal performance, long turnaround times, and high costs.
- We previously identified a serum miRNA signature, including miR-122 and miR-885, with potential for ACR diagnosis and prognosis and avoidance of immunosuppression therapy (IST) over treatment (clinicaltrials.gov/study/NCT00135694 and NCT00531921).
- We report here the analytical validation and clinical feasibility of a one-step miRNA RT-qPCR assay (HepatoTrack™) developed from this miRNA signature into a CLIA-validated lab-developed test.

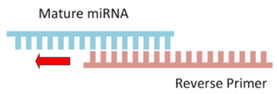
Methods

miRNA Targets

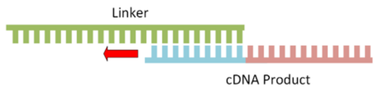
Targets	miRNA Function
hsa-miR-122-5p	Hepatocyte specific and makes up about 70% of all miRNAs in the hepatocyte. Elevated in serum sooner than ALT and to return to normal earlier after successful therapy.
hsa-miR-885-5p	Influences immune cell activation, inflammatory responses, and tissue injury regulation, all of which are critical to the development of allograft liver rejection.
hsa-miR-23a-3p	Endogenous control in blood-based samples.
cel-miR-39	Exogeneous spike-in control.

One-Step RT-PCR for miRNA Detection

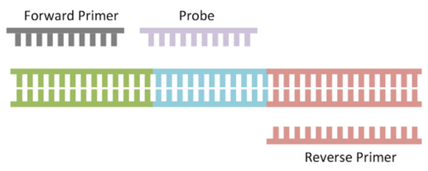
Step 1. cDNA Synthesis using Gene Specific Primer



Step 2. Annealing and Extension using Gene Specific Linker



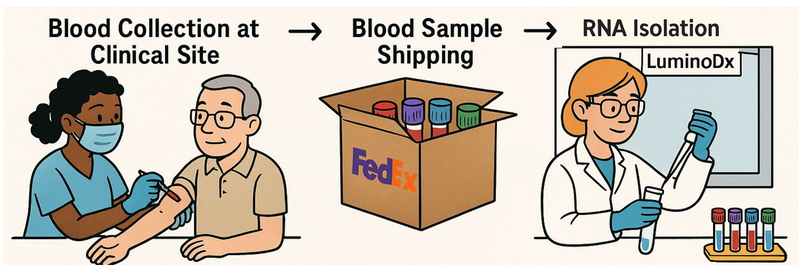
Step 3. Quantative PCR using Gene Specific Probe

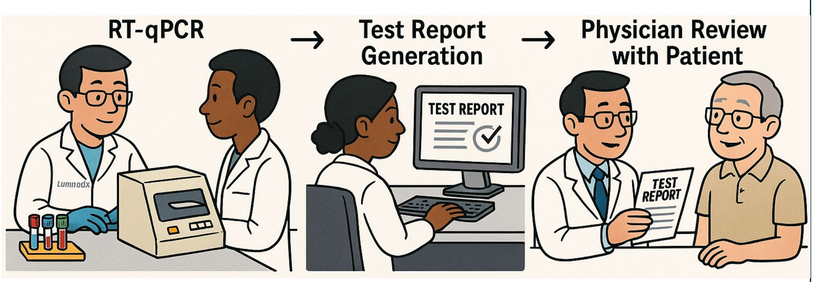


The LuminoDx miRapid™ miRNA detection assay offers a single setup, single-plex or multiplex RT-qPCR solution for analyzing miRNAs.

The high accuracy reagent system delivers consistent, sensitive and reproducible results.

Sample Processing Workflow (TAT: 24-48 Hours)





Patient Demographics

	All Patients	Training Cohort	Test Cohort
Patient Number	84	47	37
Age at Transplantation (Year)	23-72 Average 55.1	35-72 Average 57.0	23-69 Average 52.9
Gender	M (54), F (30)	M (32), F (15)	M (22), F (15)
Race	White (65), Black or African American (12), Others (7)	White (38), Black or African American (6), Others (3)	White (27), Black or African American (6), Others (4)
Days Between Liver Biopsy and Transplantation Date	36-2177 Average 402	36-1670 Average 183	181-2177 Average 681
Days Between Longitudinal Blood Draws	1-716 Average 150	1-91 Average 37	64-716 Average 293
ACR Status	No-ACR (54) ACR (31)	No-ACR (30) ACR (17)	No-ACR (23) ACR (14)

Results

Algorithm Development for ACR Prediction

1. Data Normalization: Both miR-122 and miR-885 were normalized with miR-23a.
2. Data Processing: The longitudinal changes for each gene were calculated
3. Algorithm Development: A linear regression model was developed using two predictor variables: miR122 Change and miR885 Change.

The HPS was calculated and optimized for the balanced accuracy and PPV. The linear models were fitted, and the coefficients were determined. The cutoff =2 was determined based on the balance between PPV and NPV curve.

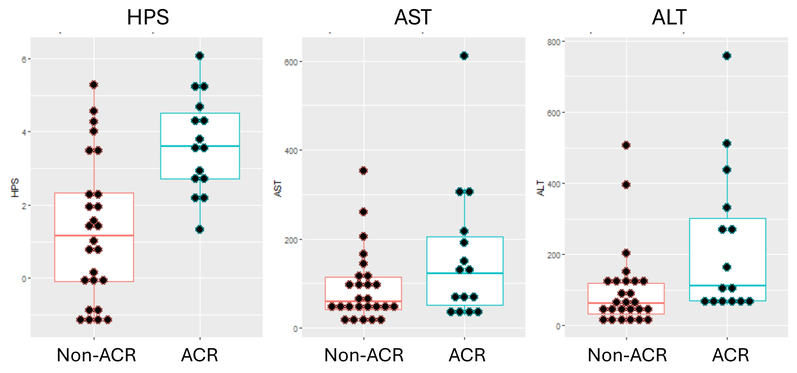
Analytical Performance

	Results
Analytical Accuracy	100%
Precision	< 10% CV
Limit of Blank (Ct Value)	miR-122: 34.89 miR-23a: 36.80 miR-885: 40.00 miR-39: 39.43
Limit of Detection (Ct Value)	miR-122: 34.12 miR-23a: 34.87 miR-885: 39.42
Lower Limit of Quantitation (Ct Value & RNA Template Copy Number)	miR-122: 32.49 (12000 Copies) miR-23a: 35.36 (1200 Copies) miR-885: 26.53 (12000 Copies)
Upper Limit of Quantitation (Ct Value & RNA Template Copy Number)	miR-122: 17.08 (24,000,000 Copies) miR-23a: 19.12 (24,000,000 Copies) miR-885: 14.11 (24,000,000 Copies)

Clinical Performance

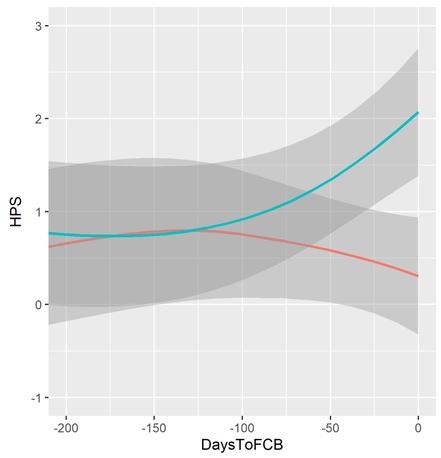
	Training Cohort	Test Cohort
True Positive (ACR)	13	13
False Positive	9	6
False Negative	4	1
True Negative (non-ACR)	21	17
Accuracy	0.7234	0.8108
Sensitivity	0.7647	0.9286
Specificity	0.7	0.7391
Pos Pred Value	0.5909	0.6842
Neg Pred Value	0.84	0.9444

HepatoTrack Shows Stronger Correlation with ACR Status Than Liver Function Tests



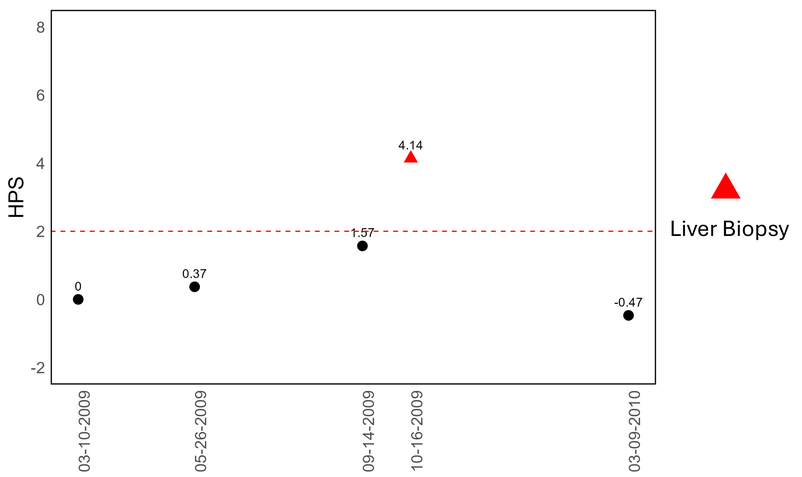
p = 0.0002738 *p* = 0.09325 *p* = 0.01541
** Wilcoxon Rank-Sum Test*

HepatoTrack Demonstrates Predictive Value of ACR



Retrospective longitudinal trajectory of HepatoTrack miRNA signature as a function of time before liver biopsy. The LOESS-smoothed averages and 80% confidence bands of signature for the ACR group (green) and the non-ACR group (red) are shown, looking backward from the time of biopsy (day 0). The y-axis denotes the HPS score, and the x-axis denotes days relative to For Cause Biopsy (FCB).

Case Study: One Patient Underwent ISW



- Patient underwent ISW and failed ISW during dosing tapering.
- ACR was confirmed with liver biopsy and elevation observed **32 days** prior to the confirmed liver biopsy.

Conclusion

- Non-invasive monitoring of allograft liver dysfunction and ACR addresses a significant unmet medical need enabling personalized care and improved management of IST.
- HepatoTrack offers simpler, faster, more cost-effective, and potentially superior performance compared to dd cfDNA or PBMC RNA assays.
- Additional independent validation further demonstrated its clinical validity.

Reference: Shaked A, Chang BL, Barnes MR, Sayre P, Li YR, Asare S, et al. An ectopically expressed serum miRNA signature is prognostic, diagnostic, and biologically related to liver allograft rejection. Hepatology 2017;65:269–280.

Acknowledgement/Funding: The clinical samples were form the study CTOT03 (NCT00531921) which we collected biological samples at fixed timepoints and at FCLB during the first year post-T.