

Performance Evaluation of DBS DNA Extraction Methods in PCR Based Newborn Screening Assays

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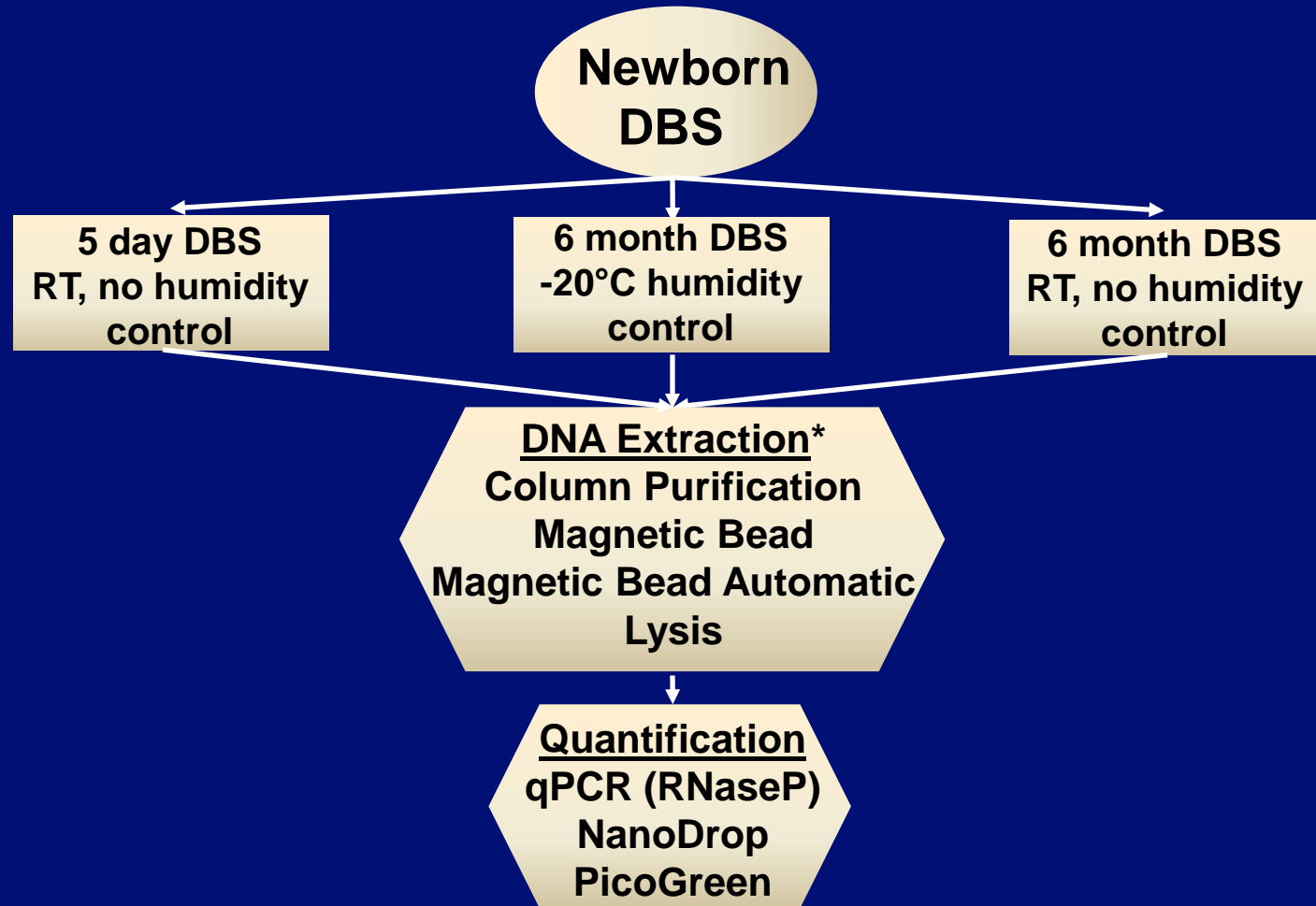
NCEH, CDC

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DBS DNA Extraction Study Goals

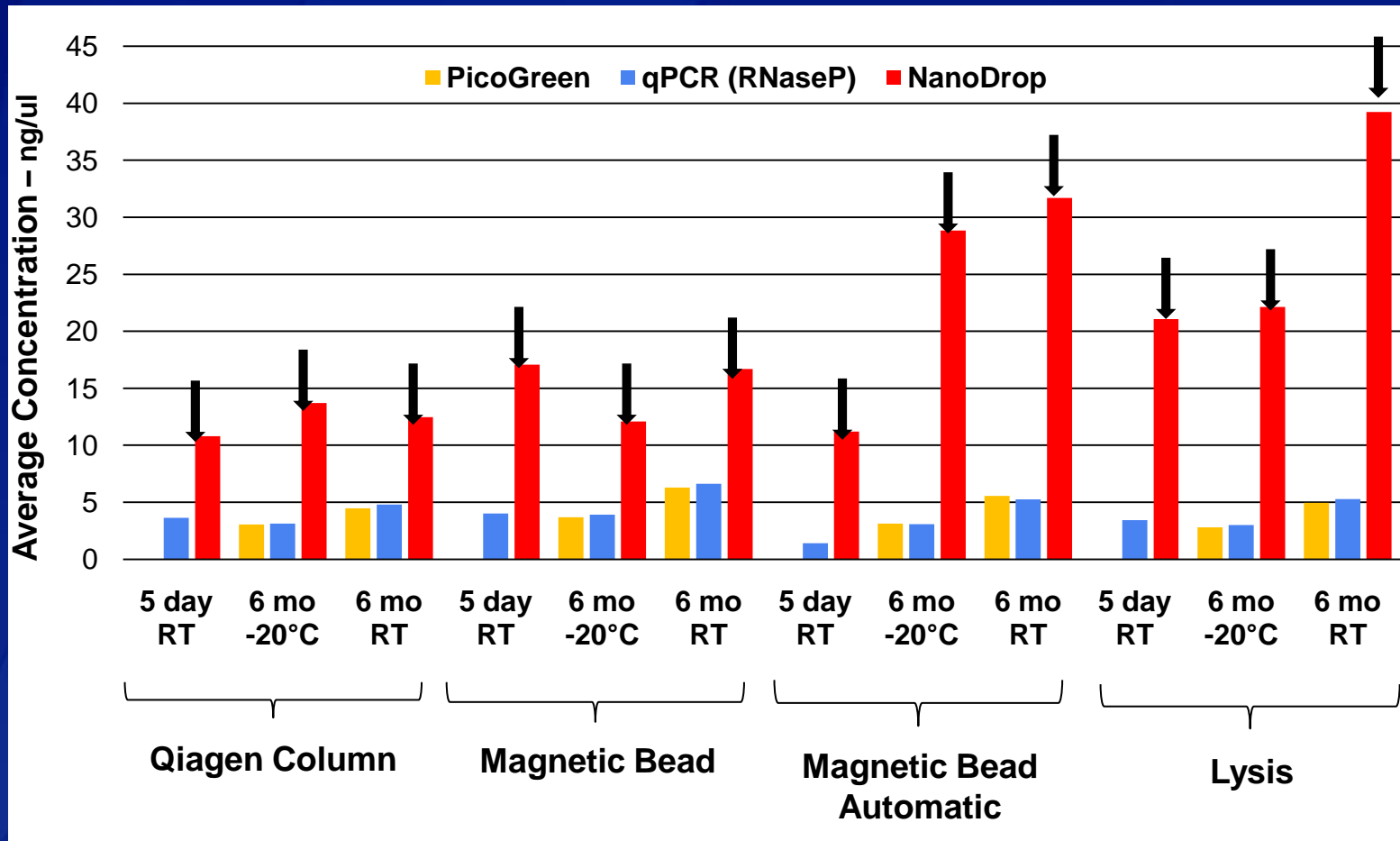
- ❑ Determine utility of DNA quantitation methods with DBS DNA**
- ❑ Examine performance of extracted DNA in assays used in NBS laboratories**
- ❑ Evaluate the performance of DNA extracted from 10 year old DBS stored in different conditions**
- ❑ Conduct first collaborative study of the NBS Molecular Network**

Evaluation of DBS DNA Extraction Methods



* DNA was extracted from one 3mm punch

DBS DNA Quantitation Methods: PicoGreen, NanoDrop and qPCR

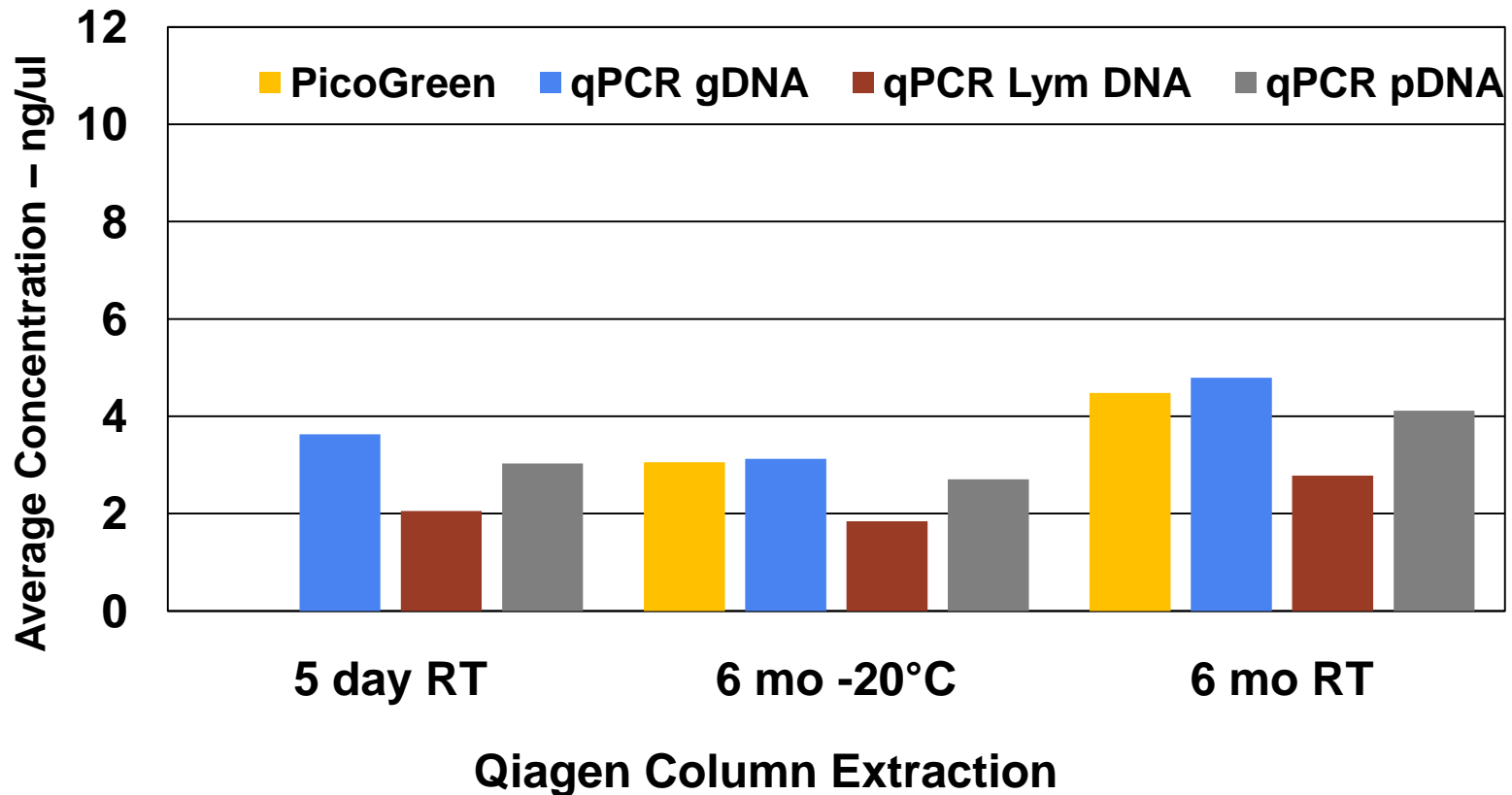


5 Day DBS were not quantitated using PicoGreen due to limited DNA

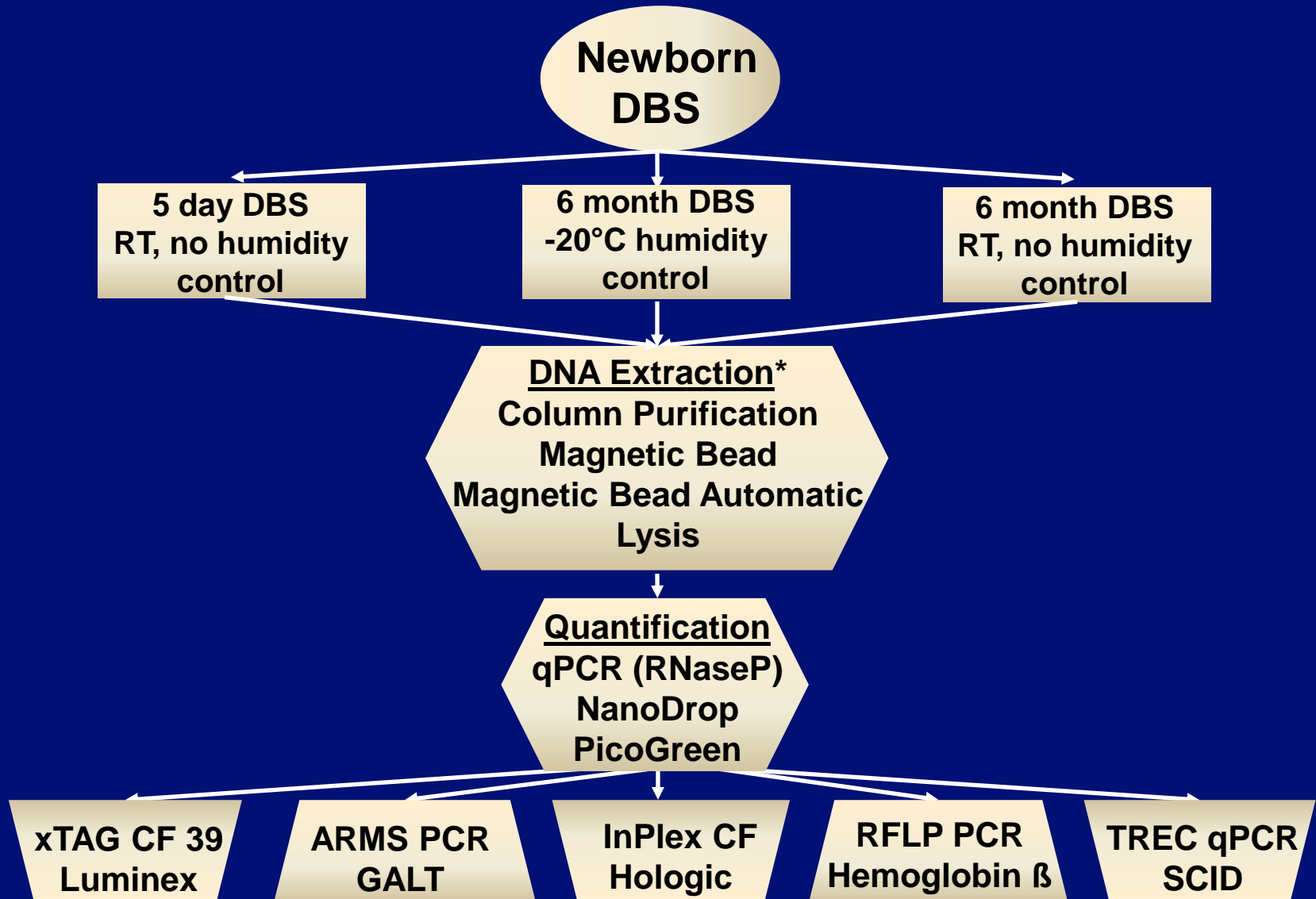
Average DNA Yield (ng) from each Extraction Method

Extraction method	Sample	PicoGreen	qPCR gDNA	NanoDrop
Column	5 day RT	N/A	218	648
	6 mo -20°C	183	188	821
	6 mo RT	269	288	711
Mag Bead	5 day RT	N/A	242	1,015
	6 mo -20°C	221	234	725
	6 mo RT	378	397	1,091
Mag Bead Auto	5 day RT	N/A	85	671
	6 mo -20°C	188	185	1,711
	6 mo RT	334	315	1,911
Lysis	5 day RT	N/A	206	1,105
	6 mo -20°C	169	180	1,123
	6 mo RT	297	317	2,354

DBS DNA Quantitation qPCR: Using Different Standard Curve Materials



Evaluation of DBS DNA Extraction Methods



* DNA was extracted from one 3mm punch

DBS Extracted DNA Performance in Molecular NBS Assays

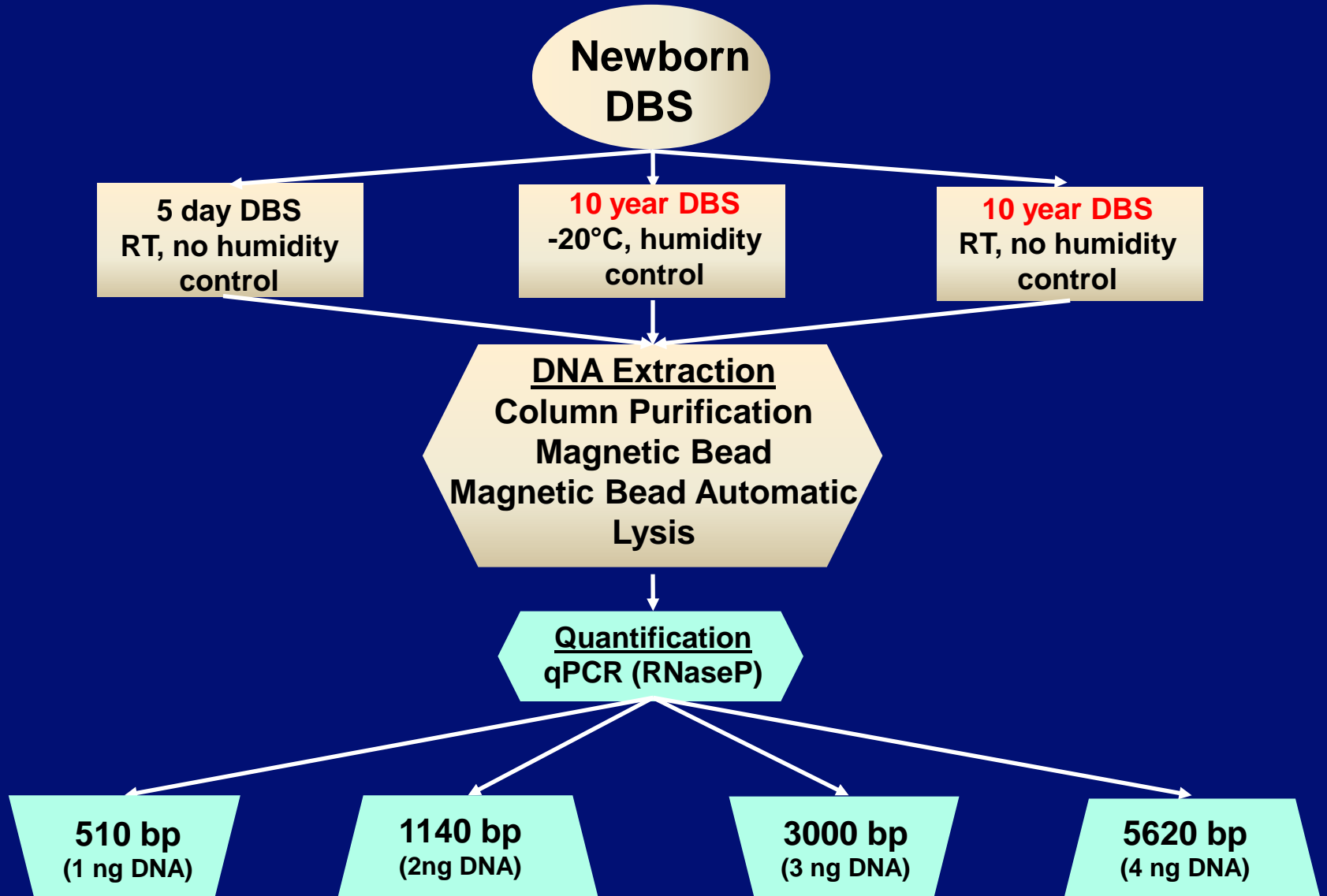
	Age/Storage	Col	Mag	MagA	Lvs
xTAG CF 39 kit v2 Failures (%)	5 day RT	0	10 (50)	0	0
	6 mo -20°C	0	16 (80)	6 (30)	0
	6 mo RT	1 (5)	10 (50)	7 (35)	0
ARMS GALT PCR Failures (%)	5 day RT	0	1 (5)	0	0
	6 mo -20°C	0	0	20 (100)	0
	6 mo RT	0	0	20 (100)	0
Inplex CF Failures (%)	5 day RT	0	(5)	17 (85)	2 (10)
	6 mo -20°C	4 (20)	4 (20)	20 (100)	4 (20)
	6 mo RT	0	0	11 (55)	0
PCR-RFLP HbB Failures (%)	5 day RT	0	0	0	1 (5)
	6 mo -20°C	0	0	0	0
	6 mo RT	0	0	0	0
qPCR-TREC # TREC (Cq)	5 day RT	362 (31.6)	342 (31.6)	252 (31.9)	242 (32.2)
	6 mo -20°C	319 (31.8)	433 (31.2)	175 (32.7)	244 (32.2)
	6 mo RT	365 (31.4)	444 (31.0)	204 (32.3)	448 (31.0)

Assay failures denoted in red and reduced TREC amplification denoted in blue.

Study Conclusions - Part 1

- ❑ DBS DNA quantitation with the NanoDrop overestimates quantity and is not suitable for DBS DNA
- ❑ qPCR performed with different standard curve sources does not perform the same and care should be taken when comparing yields
 - LYM DNA and pDNA source - 0.59 fold & 0.87 fold lower than gDNA respectively
- ❑ Column based and lysis DNA extractions performed consistently better in all assays

Evaluation of PCR Amplification in DBS



DBS PCR Amplification Results

Age/ Storage	Extraction	510 bp Failures (%)	1140 bp Failures (%)	3000 bp Failures (%)	5620 bp Failures (%)
5 Day RT (N=5)	Col	0	0	0	0
	Mag	3 (60)	0	1 (20)	0
	MagA	1 (20)	0	1 (20)	0
	Lys	0	0	0	0
10 Year -20°C (N=20)	Col	1 (5)	1 (5)	0	0
	Mag	1 (5)	0	1 (5)	0
	MagA	1 (5)	0	12 (60)	7 (35)
	Lys	0	0	1 (5)	2 (10)
10 Year RT (N=20)	Col	0	0	1 (5)	20 (100)
	Mag	3 (15)	1 (5)	20 (100)	20 (100)
	MagA	1 (5)	1 (5)	15 (75)	20 (100)
	Lys	1 (5)	0	20 (100)	20 (100)

Study Conclusions - Part 2

- ❑ **Storage conditions of archival DBS does not affect amplification of smaller fragments (≤ 1.1 kb)**
- ❑ **Storage conditions of archival DBS affects amplification of larger fragments (≥ 3 kb)**
 - **RT conditions: 3 kb fragments amplified from DBS extracted using a column method**
 - **-20°C conditions: 5.6 kb fragment routinely amplified from DBS for all extraction methods**

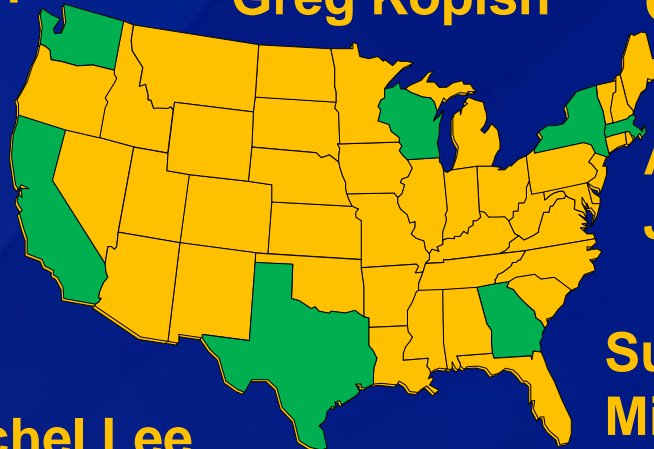
A Successful First Collaborative Study

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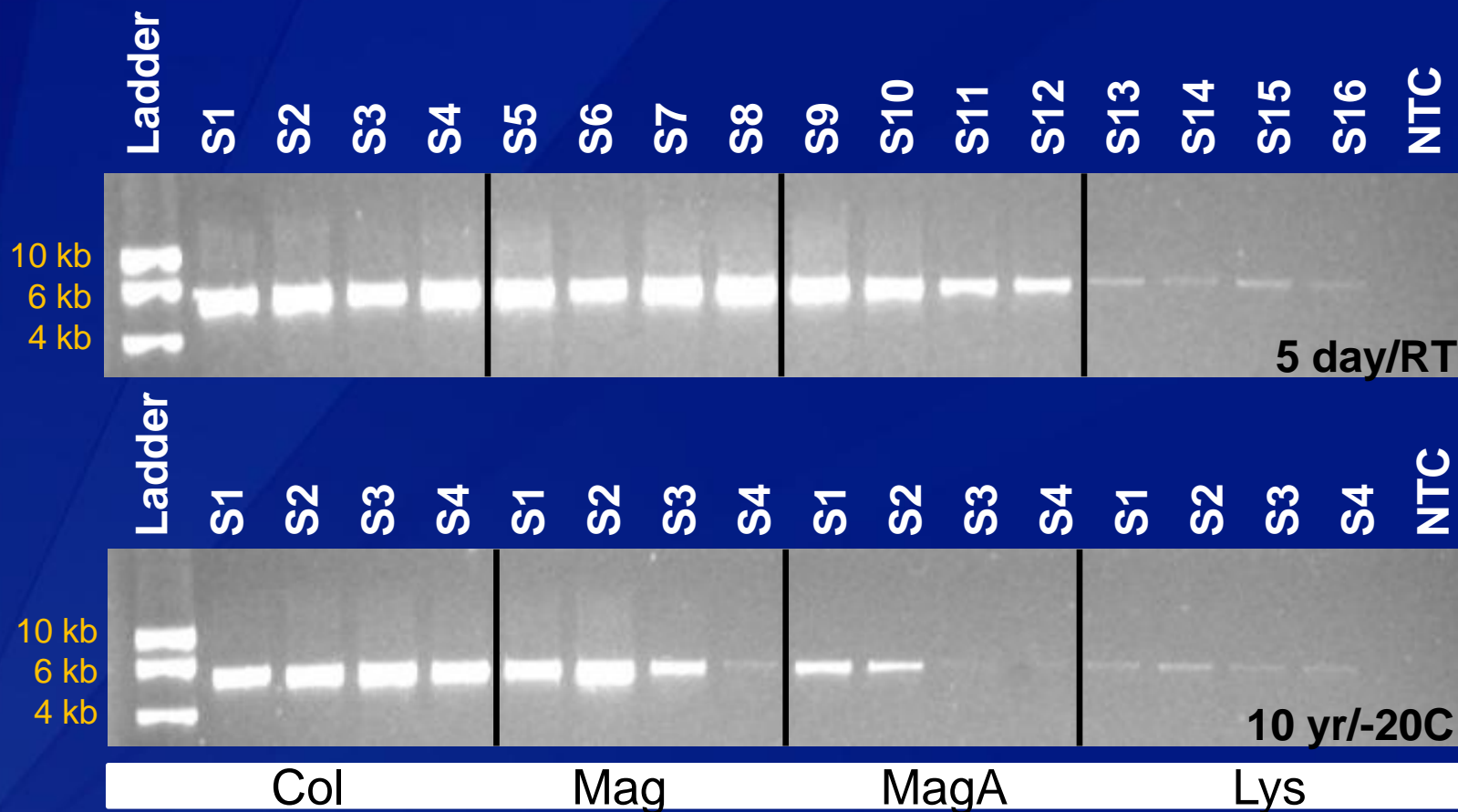
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Stanimila Nikolova
Sean Mochal
Daniel Turner
Francis Lee



Amplification of 5.6 kb Product

5 day RT and 10 yr -20°C DBS



Amplification of 1.1, 1.9 and 3 kb Products 10 yr DBS RT

