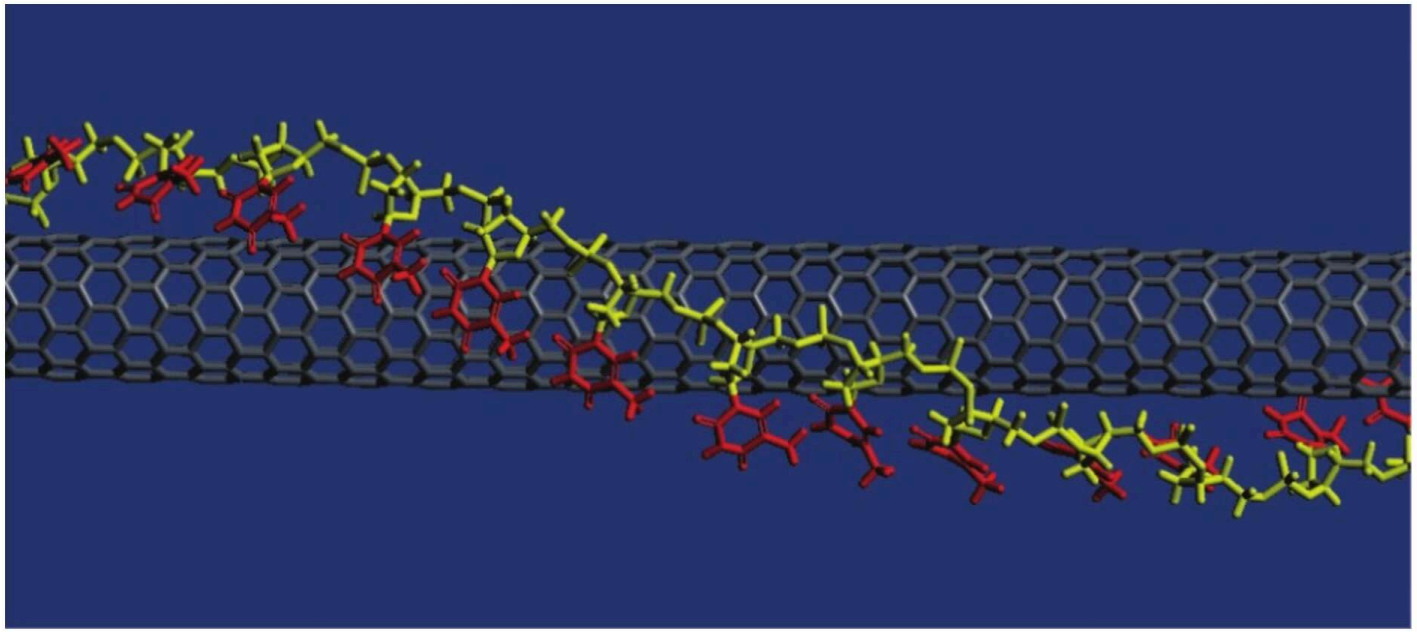
Life Magnetics

Non-Confidential Technology Overview

Summary

- Developed Carbon-based RNA isolation solution
- Extremely selective for single stranded nucleic acids, no detectable DNA contamination by PCR with optimization
- Compatible with Trizol, no chloroform, no precipitation, <15 min, better yield and purity, better sequencing results (best selling application)
- Long term goal is shipping and storage applications. Room temperature shipping with >30 days stability.

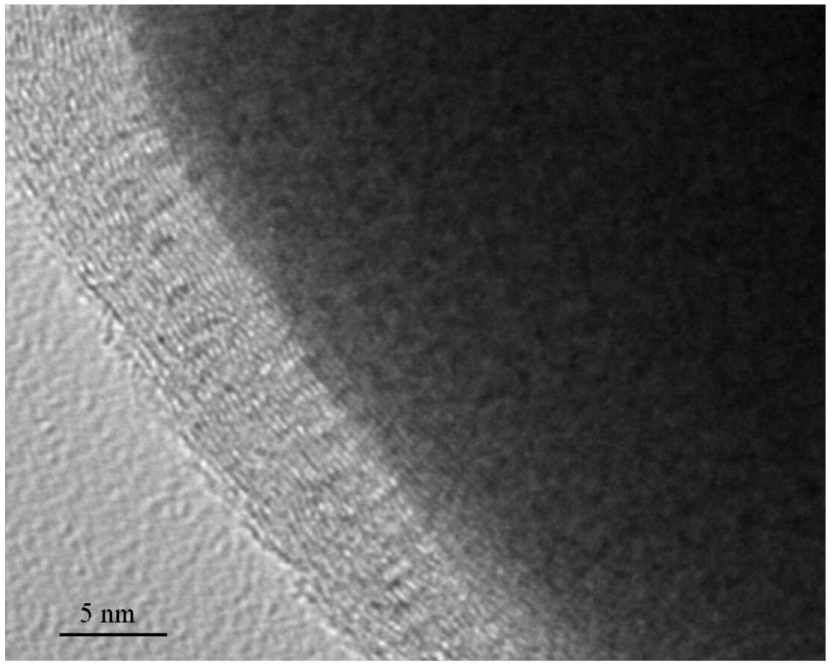
Theoretical Basis



Single Stranded Nucleic Acid Binding to Carbon Discovered 2003

Zheng, M. *et al.* DNA-assisted dispersion and separation of carbon nanotubes. *Nat. Mater.* **2,** 338–342 (2003).

Development Efforts



TEM image of actual product, layers are individual atomic layers of carbon, core is metal

Development activities:

- Engineered Carbon with affinity for RNA
- Engineered buffers and protocols for carbon surface

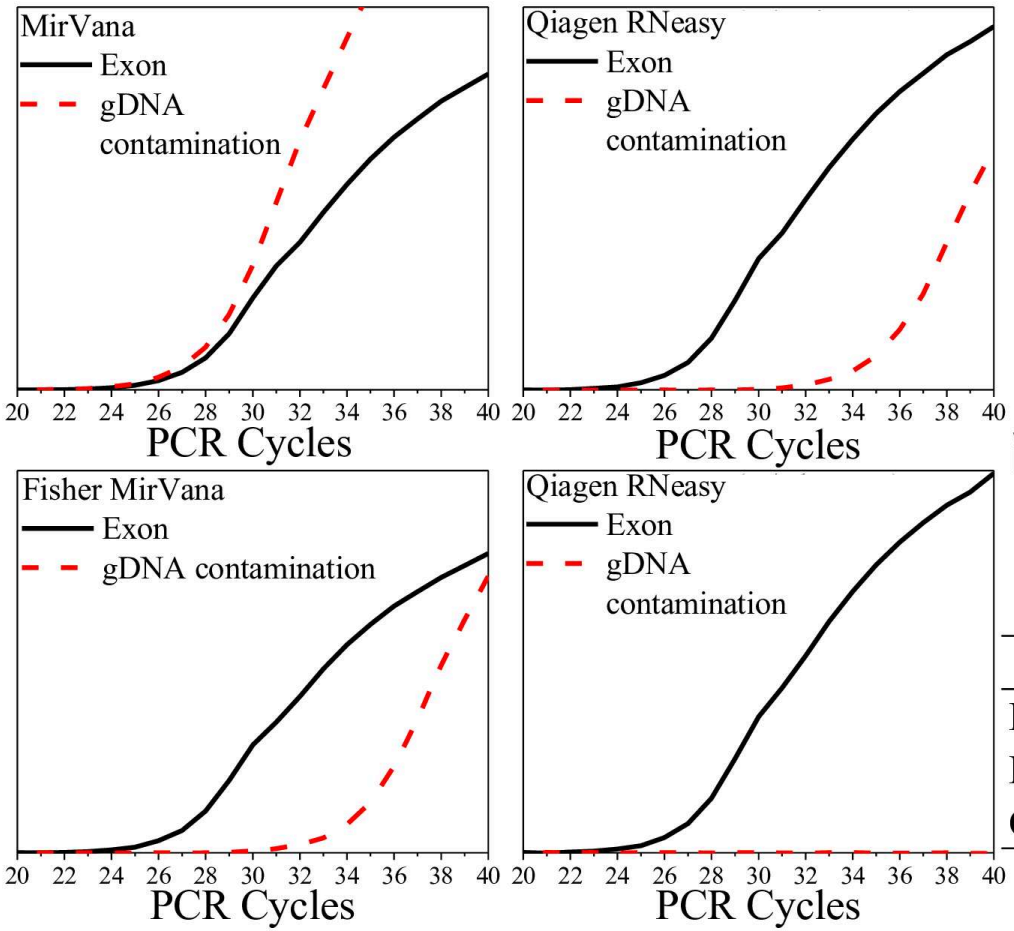
Developed with support from the National Science Foundation Award #1745992

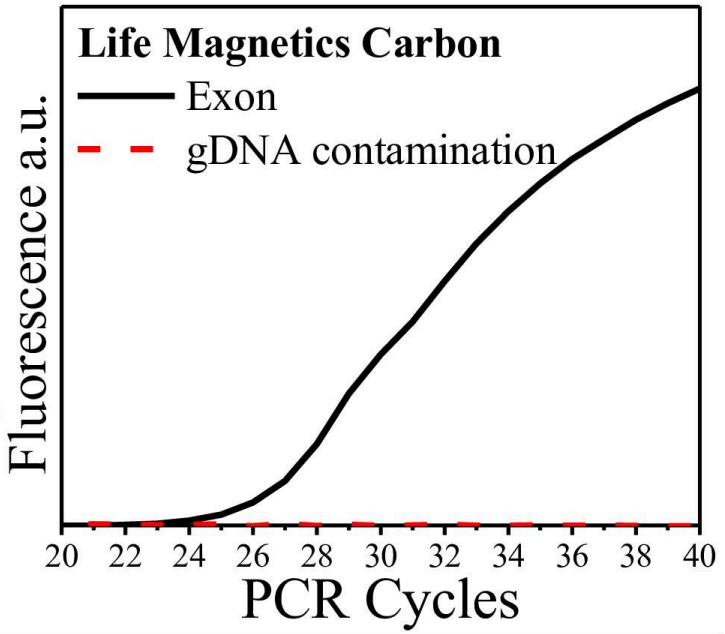
Results: Mouse Liver

No DNase cleanup

DNase cleanup

Fisher and Qiagen products leave considerable DNA contamination





	Yield (ug/mg)	RIN#	DNA?
LM	**6.19 ± 1.17**	**9.4**	**No**
Fisher	4.11 ± 0.5*	8.5	Yes, a lot
Qiagen	2.91 ± 0.5	8.7	Yes, trace

Figure 1: Comparison of RNA yield and purity obtained by the Life Magnetics kit, Qiagen RNeasy, and Fisher MirVana for mouse liver tissue. *Results with the Fisher kit are an artifact, see objective 4.

Results: Automated COVID-19 testing

Life Magnetics, Inc on Hamilton STAR

Hamilton LM TRIzol protocol	S gene	N Gene	MS2	ORF1ab	Proteinase K and/or Carrier RNA used
CVC 39510 LM	30.37	32.48	27.31	30.43	No
CVC 39527 LM	Undetermined	Undetermined	26.23	Undetermined	No
CV 035888 LM	Undetermined	Undetermined	26.18	Undetermined	No
CVC 4128C LM	32.03	33.33	25.77	32.01	No
CV 041276 LM	33.39	32.42	26.96	31.79	No
CVC 39511 LM	32.32	32.13	25.63	Undetermined	No
CVC 36733 LM	16.51	17.99	34.33	16.93	No

100 ul Sample, half the volume reduces VTM use

Table 4. Hamilton-Omega silica-beads used for isolating SARS-CoV-2 viral RNA from Nasopharyngeal swab in VTM. The process depends on the use of expensive carrier RNA and enzymatic digestion with Proteinase K before processing the viral samples.

Omega Biotek on Hamilton STAR

Hamilton Omega protocol	S gene	N Gene	MS2	ORF1ab	Proteinase K and/or Carrier RNA used
CVC 39510 Omega	29.98	32.36	27.34	30.72	Yes
CVC 39527 Omega	Undetermined	Undetermined	26.16	Undetermined	Yes
CV 035888 Omega	Undetermined	Undetermined	26.21	Undetermined	Yes
CVC 4128C Omega	29.71	34.09	27.56	32.06	Yes
CV 041276 Omega	33.60	33.64	28.21	Undetermined	Yes
CVC 39511 Omega	30.78	32.75	27.62	31.95	Yes
CVC 36733 Omega	14.78	16.96	31.07	15.10	Yes

200 ul Sample

Table 3. Hamilton-LM Carbon-beads used for isolating SARS-CoV-2 viral RNA from Nasopharyngeal swab in VTM. The process don't need any expensive carrier RNA or enzymatic digestion with Proteinase K before processing the viral samples.

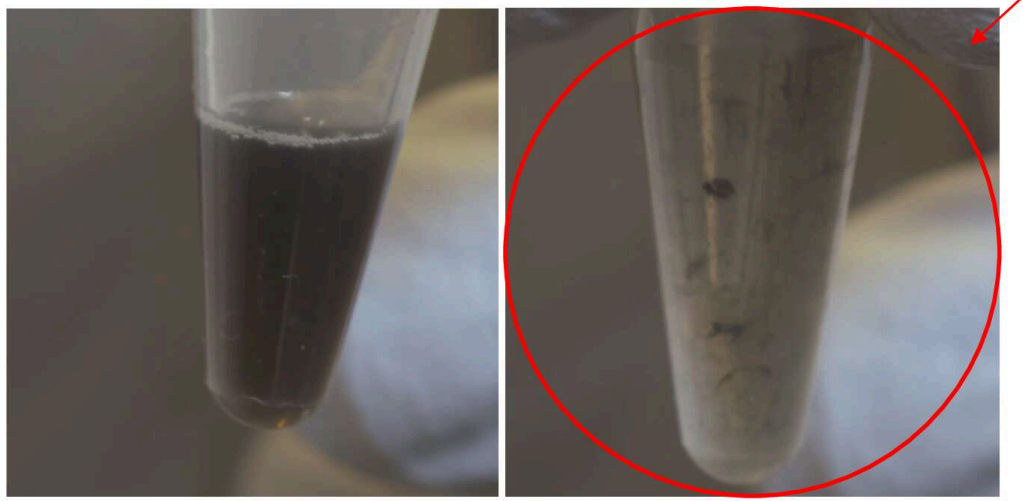
Results: Clinical Data, COVID-19

Results from COVID-19 patient samples

Saliva sample ID	S gene	N gene	MS2	ORF1ab	Call
VTM-RMD-0136	Undetermined	Undetermined	24.51	Undetermined	Negative
VTM-RMD-0144	38.56	Undetermined	25.75	Undetermined	Negative
VTM-RMD-0152	Undetermined	Undetermined	26.38	Undetermined	Negative
VTM-RMD-0145	Undetermined	Undetermined	29.41	Undetermined	Negative
VTM-RMD-0130	Undetermined	Undetermined	24.97	34.89	Negative
VTM-RMD-0138	Undetermined	Undetermined	24.24	Undetermined	Negative
VTM-RMD-0146	Undetermined	Undetermined	27.40	Undetermined	Negative
VTM-RMD-0131	39.07	Undetermined	27.20	Undetermined	Negative
VTM-RMD-0139	Undetermined	Undetermined	26.99	Undetermined	Negative
VTM-RMD-0147	36.76	35.91	23.68	37.84	Positive
VTM-RMD-0132	39.95	Undetermined	27.35	Undetermined	Negative
VTM-RMD-0140	Undetermined	Undetermined	25.02	Undetermined	Negative
VTM-RMD-0148	36.90	38.66	22.73	36.94	Positive
VTM-RMD-0133	18.70	34.05	26.70	Undetermined	Positive
VTM-RMD-0141	Undetermined	Undetermined	25.89	Undetermined	Negative
VTM-RMD-0149	Undetermined	Undetermined	30.62	Undetermined	Negative
VTM-RMD-0134	39.08	Undetermined	24.87	Undetermined	Negative
VTM-RMD-0142	35.39	36.87	21.40	35.67	Positive
VTM-RMD-0150	Undetermined	Undetermined	27.30	Undetermined	Negative
VTM-RMD-0135	Undetermined	Undetermined	24.47	Undetermined	Negative
VTM-RMD-0143	Undetermined	Undetermined	26.93	Undetermined	Negative
VTM-RMD-0151	Undetermined	Undetermined	26.39	Undetermined	Negative
NEC	Undetermined	Undetermined	30.18	Undetermined	Negative

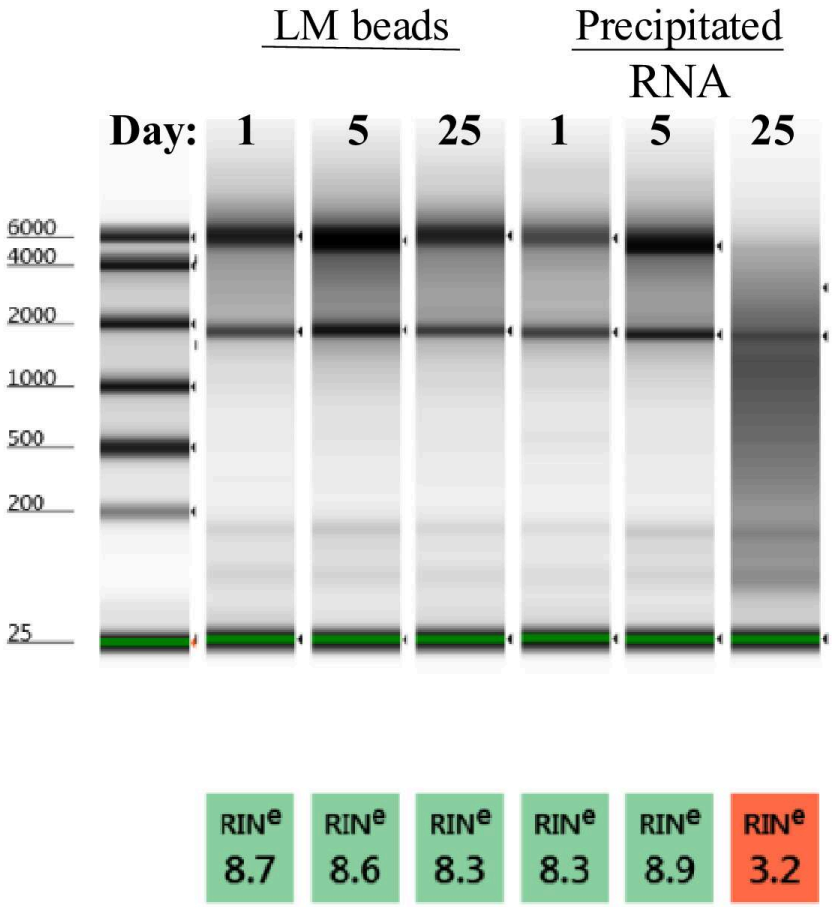
RNA Shipping

Once stabilized in aggregates as shown below, RNA is stabilized against RNase activity



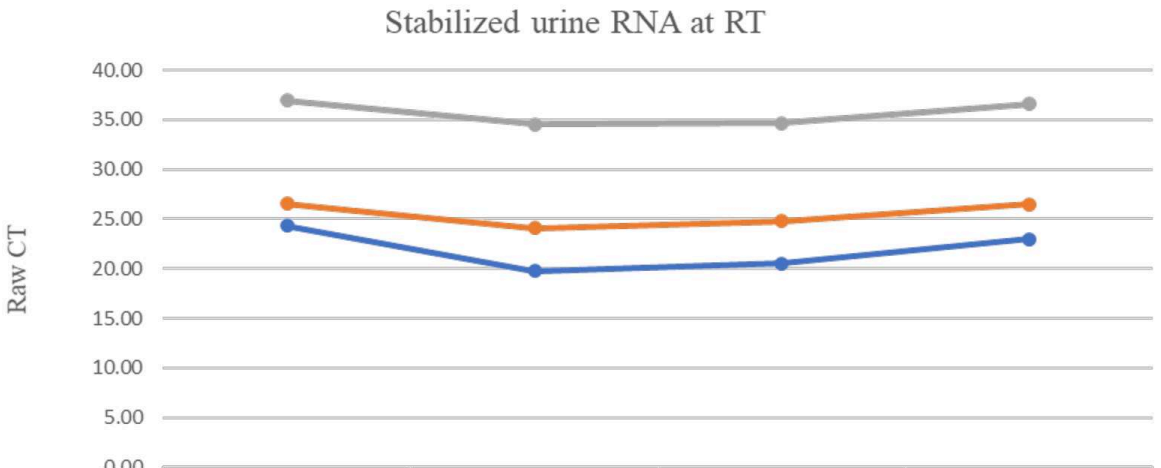
RNA capture clearly visible and customers love that!

No RNA bound RNA bound



RNA Shipping - Urine

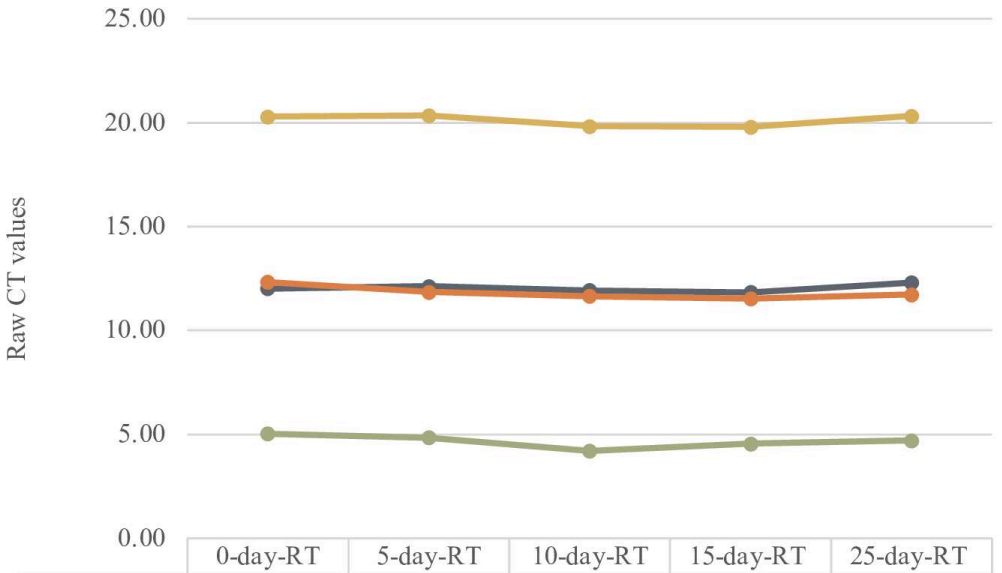
One-step method, RT shipping and storage



	0-day RT	2-day RT	4-day RT	6-day RT
18S rRNA	24.32	19.76	20.55	22.98
B-actin	26.55	24.09	24.78	26.50
GAPDH	36.96	34.57	34.68	36.61

Just add urine, seal, and ship!

2-Step Method, RT shipping and storage



	0-day-RT	5-day-RT	10-day-RT	15-day-RT	25-day-RT
Actin	12.02	12.13	11.92	11.83	12.30
PUM1	12.33	11.85	11.65	11.54	11.73
18srRNA	5.03	4.85	4.21	4.55	4.71
TBP	20.28	20.35	19.83	19.80	20.32

2 step collection: requires adding urine to vial with enzymes to lyse, then putting vial in magnetic holder and pouring out liquid and adding ethanol, then sealing and shipping.

Simple Chemistry

- RNA binding – 50 mM $CaCl_2$, 40-50% ethanol
- Washing – EDTA and Tris-EDTA as a wash to remove Ca^{2+}
- Elute in water

- No carrier RNA! Tested with carrier RNA from Sigma, Qiagen, and Omega, there is no need to include this
- Compatible with most lysis buffers. Excellent performance with phenol based lysis buffers with no phenol carryover. Proteinase K lysis buffers benefit from Ca^{2+}

User review

“It worked really well! I obtained about 10-20x as much RNA as I typically do, which was great to see. The 260/280 was 1.98 and the 260/230 was above 2 as well. It also was a very quick protocol, which was amazing too. When I was working with ~20 samples using our old method it would've taken about an entire day just to get RNA that was DNase treated.”

Christopher Mataczynski graduate student at WSU

User review

“We had positive news for your kit in the isolation of RNA from cell lines (good quality RNA with an overall RNA yield similar to Qiagen). Your carbon-based beads also bound RNA more efficiently in the low molecular weight range compared to Qiagen (RNeasy kit).”

- RNA Diagnostics

Life Magnetics

Thank you!

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