Rapid Ribonuclease Detection Lateral Flow (Strip) Test

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Abstract

Production of therapeutic and diagnostic RNA requires strong Manufacturing Controls and Quality Management Systems. The expansion of therapeutic RNA manufacturing in recent years has increased the need for improved process control tools. One of the most uniquely important aspects of RNA manufacturing is the need for ribonuclease (RNase) control throughout the entire production process. Even trace levels of ribonuclease contamination can compromise RNA quality. Accidental ribonuclease contamination can damage or destroy highly valuable RNA product. Unfortunately, current RNase detection products require several hours to perform using expensive laboratory instrumentation by highly skilled operators.

We have created a unique, simple, and convenient lateral flow assay (LFA) for on-site detection of ribonucleases in RNA vaccines, therapeutics and diagnostic devices. The speed, simplicity and sensitivity of our test far exceed those of any other commercial ribonuclease detection kit. Our patented LFA product is portable, allowing testing to be performed anywhere it is needed throughout the manufacturing facility.

Our ultrasensitive LFA can detect less than 0.1 picogram of RNase A in less than 20 minutes. The speed of our test permits rapid verification of the production environment and supply-chain materials in real-time before, during, and after the manufacturing process. It can be used to provide a rapid qualitative visual readout (requiring no equipment) or, when used with our portable strip reader, it can provide a traceable and quantitative result. The robust design and simple workflow allow the test to be reliably performed by manufacturing staff possessing limited scientific training. Our self-contained test can be used to detect RNase present in liquids or on hard surfaces. Swabs provided with the test allow convenient sampling of hard surfaces, which is difficult to perform with current RNase testing methods.

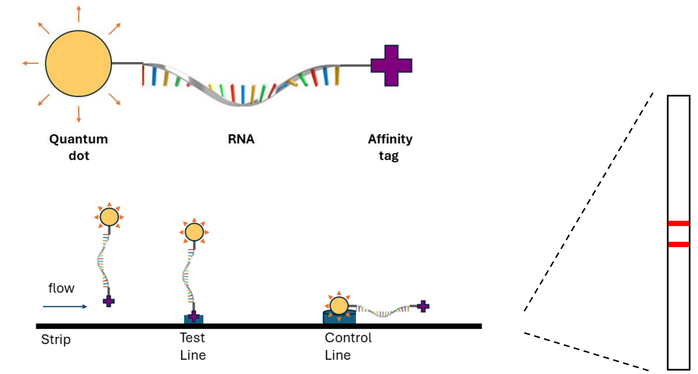
Introduction

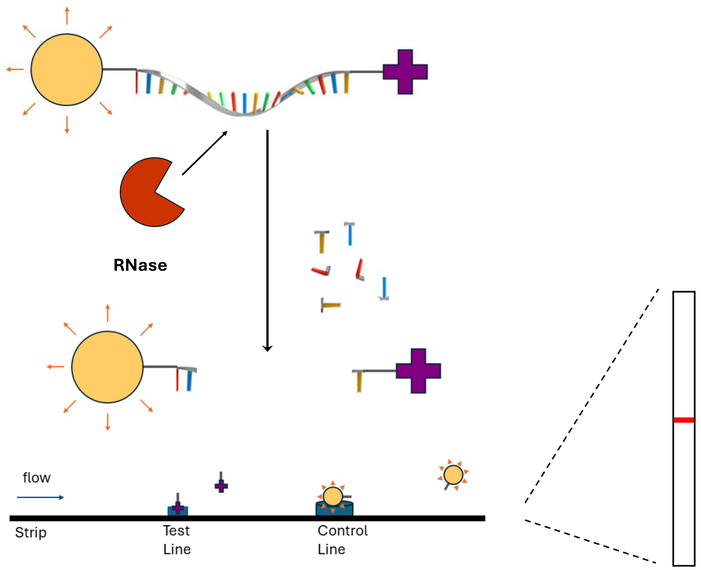
The importance and impact of therapeutic and diagnostic RNA have been increasing exponentially over the past two decades. Seminal improvements in the design, delivery, and manufacturing processes of RNAs have created exciting new possibilities for their expanded use in a variety of *in vivo* models. More than 10 RNA drugs have been approved by the FDA in the past 10 years. Consequently, pharmaceutical and biotechnology companies are currently ramping up their efforts to develop RNAs for a variety of important therapeutic applications. For example, small interfering RNAs, siRNAs, are being clinically developed as therapeutics to fight diseases such as cancer and macular degeneration. Larger mRNA molecules are also undergoing intense clinical development efforts, since mRNAs have been successfully used as vaccines against infectious agents and are in clinical development for cancer immunotherapy applications. RNA molecules are also being developed for important clinical diagnostic applications as well.

The key to generating safe and effective RNA vaccines/therapeutics is careful attention to quality control. The 'Achilles Heel' in many RNA-related endeavors are ubiquitous RNases that are generated and secreted by all organisms (and generally do not require divalent cation cofactors like their DNase counterparts). RNA manufacturing processes for FDA-regulated applications such as vaccine production require the exclusive use of RNase-free facilities. Therefore, RNA experiments are challenging due to the invasive nature of RNases that can surreptitiously be introduced and significantly influence the quality or effectiveness of the outcome. We have developed an effective, easy to use, and highly sensitive 'early warning' system to detect RNase contamination in manufacturing environments and supply chain components. Our system, described in this poster, has unsurpassed speed and sensitivity which provide a means to proactively prevent RNase contamination issues before they happen, as well as easily troubleshoot RNase problems in solutions, etc., to rapidly solve problems when they arise.

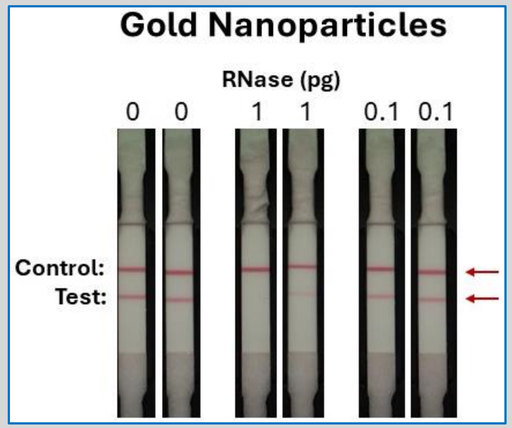
Assay Principle

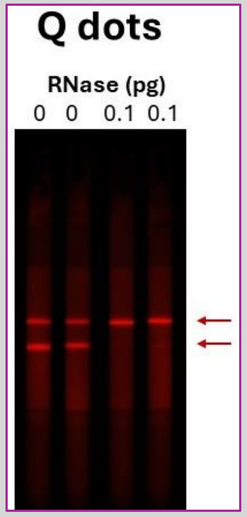
Our simple test utilizes RNA oligonucleotides coupled to highly fluorescent quantum dots on one end and an affinity tag on the other. Intact RNA permits the quantum dots to be tethered to the test line, thus producing a strong test line signal when RNase is absent from test samples. These oligonucleotides can be mixed with liquid samples and applied to sample pads at the end of a nitrocellulose membrane strip. The oligonucleotides can bind to both the test line and control line as they flow up the strip, creating a two-line visual or fluorescent signal on the strip. The test is also available in a colloidal gold nanoparticle format which permits a visual readout for the assay.





Results





Gold Nanoparticles

RNase (pg)	Test line	Control line	Ratio	Average ratio	Percent Avg. Knockdown
0	508257	772484	0.66	0.67	--
0	499811	736408	0.68		
0.1	424868	695812	0.61	0.57	**15.0**
0.1	360472	677306	0.53		
1	15901	836736	0.02	0.11	**83.6**
1	124427	648541	0.19		

Quantum (Q) Dots

RNase (pg)	Test line	Control line	Ratio	Average ratio	Percent Avg. Knockdown
0	4132	3344	1.24	1.22	--
0	3596	3020	1.19		
0.1	123	4405	0.028	0.048	**96.1**
0.1	282	4151	0.068		

In larger experiments, the RSD for Q Dots was determined to be **under 10%**.

Conclusions & Future Directions

1. Our novel quantum dot-based assay permits rapid detection of extremely low amounts of ribonuclease from samples.
2. We are developing a portable reader and application to quantify and trace amounts of RNase in therapeutic and diagnostic RNA facilities.