RNAseFLOW: A first of its kind device to detect ribonuclease contamination using a lateral flow test

Introduction:

The past decade has brought an escalating growth of important new commercial applications for RNA. RNA molecules have emerged as a preeminent and very versatile regulators of gene expression. They have, for example, a unique ability to direct enzymes to edit genomes (e.g. CRISPr) and destabilize gene products (e.g. siRNA technology). New diagnostic and therapeutic applications for RNA are continually being discovered and developed. Finally, modified mRNA nanoparticle formulations are enabling the development of new vaccines and cellular therapies with a speed and efficacy unrivaled by other processes. This newfound success has driven increased interest and efforts in many areas of RNA research. This has, of course, also opened the door to many new commercial opportunities in the RNA technology space for startups and large pharmaceutical and diagnostic companies alike. Accordingly, significant efforts are also now being directed to production scale-up and large-scale manufacturing of RNA molecules. As RNA R&D and large-scale production increases, there is a parallel increased need for care and quality control of RNA at all stages of these processes. Quality control is especially important for RNA since ribonucleases are extraordinarily abundant in the surrounding environment, RNAses are extremely stable proteins, and most RNAses act in a cofactor-independent fashion to degrade RNA. The development of tools and assays to assist in RNAse detection and RNA quality control, therefore, is paramount for this growing sector of the biotech industry.

As production scales and costs increase, close attention must be paid to strict process control and quality management. For example, manufacturing of RNA for regulated therapeutic and diagnostic applications must be performed in a certified RNAse-free environment. Current commercial methods to verify the absence of RNAse require expensive equipment and hours to perform. The cost and time required to perform RNAse testing can therefore hinder the productivity of manufacturers and researchers alike. A new rapid tool to routinely test for unwanted RNA contamination would greatly assist researchers and manufacturers. We are pleased to announce that we have developed a tool to specifically address this need.

Attogene has created a novel lateral flow test for the sensitive detection of RNAse with several important advantages over currently available RNase contamination detection tools. Our RNAseFlow assay is (1) convenient (does not require expensive equipment), (2) saves time (can be performed in 15 minutes as compared to hours), (3) is sensitive (detects picogram amounts of RNAse A), and (4) enables visual readouts which can be quantified using lateral flow strip readers. These combined advantages of our novel product will create a highly effective tool to further support the continuing innovation in RNA therapeutics and research.

Results:

Attogene has developed a first of its kind lateral flow assay to detect ribonuclease activity. This assay uses streptavidin-colloidal gold to bind to oligonucleotide substrates (DNA or RNA) containing a biotin on the 5' end and FITC on the 3'end. If ribonucleases are present, the RNA substrate will be degraded and prevent the colloidal gold from binding to the test line. In turn, a reduction in test line is observed in the presence of ribonucleases. In Figure 1 we show data that depicts the sensitivity of the RNaseFLOW assay to detect ribonuclease contamination. We added known concentrations of ribonuclease A into the reaction wells, mixed well, added running buffer solution, and added the lateral flow strip containing streptavidin conjugated colloidal gold in its conjugate pad.

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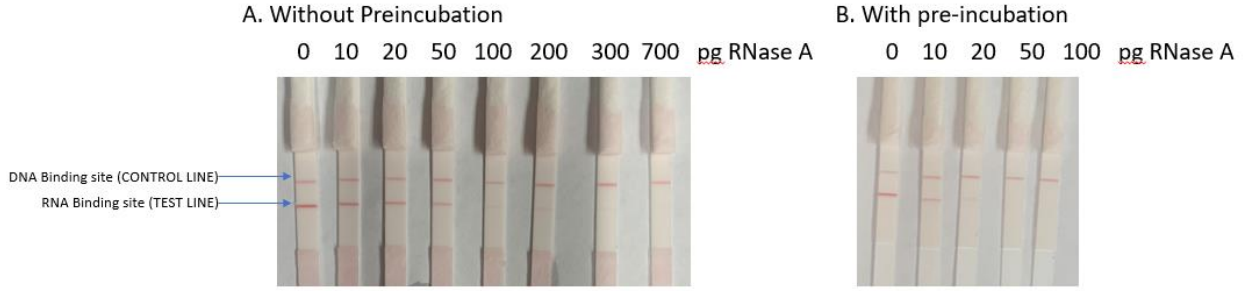


Figure 1. Ribonuclease A detection sensitivity using the RNaseFLOW test with or without preincubation. The indicated amount of RNase A was added into the RNase FLOW lateral flow running buffer and the dipsticks were added into the wells and run for 15 minutes. Visual analysis can be used to see an appreciative decrease in test line signal even as low as with 10pg of RNase A. The visual impact of this is increased when the sample is preincubated

We next evaluated the ability of the RNaseFlOW test to detect and verify the utility of RNase Inhibitors. RNAseFlOW provides an effective unit activity assay when quantifying RNase inhibitors relative strength. Figure 2 demonstrates the ability of RNase inhibitors to block RNase activity using the RNaseFLOW assay kit. It can be clearly observed that the addition of RNase Inhibitor prevents the degradation of the substrate RNA in the RNaseFlOW assay. These data indicate that the RNaseFlOW test is versatile and could also be leveraged to quantify relative strength of nucleases inhibitors.

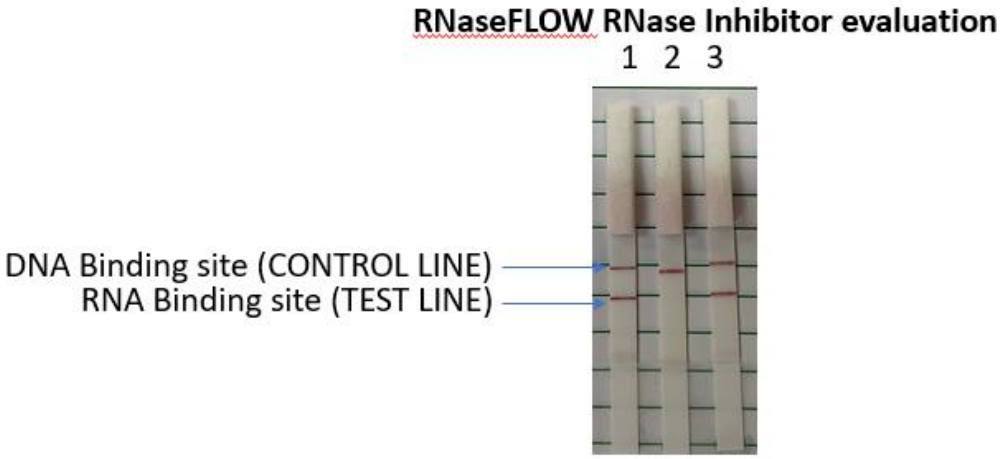


Figure 2. Evaluation of RNase inhibitor using the RNaseFLOW assay. A solution of buffer containing biotinylated RNA, biotinylated DNA oligonucleotides and streptavidin colloidal gold were mixed with running buffer (**Lane 1**) running buffer containing ribonuclease A (**Lane 2**) or running buffer containing ribonuclease A **and** ribonuclease inhibitor (**Lane 3**).

Finally, we envision that a major use of the Attogene RNaseFlOW test will be as a monitoring tool for hard surfaces in the laboratory and other facilities. Swabbing work surfaces should be regularly performed to scan for the presence of ribonucleases which could impact RNA studies and RNA

manufacturing. Regular swabbing and RNAse testing are a great way to prevent debilitating RNase outbreaks. As shown in Figure 3, the RNaseFLOW test performs excellently a swipe test configuration:

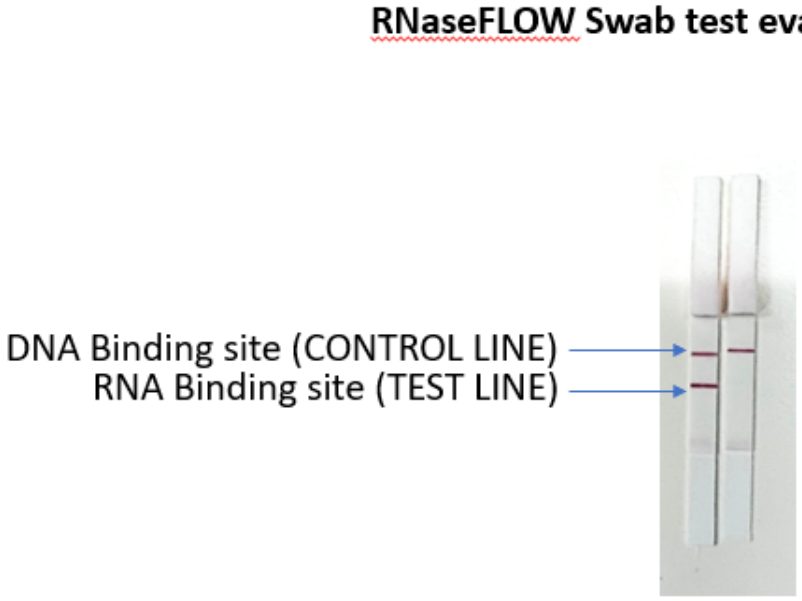


Figure 3. Swabs were added into negative control tube without RNase A (lateral flow strip to the left). or a swab was rubbed against tape with dried ribonuclease A and then placed in lateral flow assay running buffer (lateral flow strip to the right). Next, lateral flow strips were added into the wells and run for 15 minutes. The results clearly indicate that the RNaseFlOW test can be easily adapted for detecting ribonucleases found on surfaces.

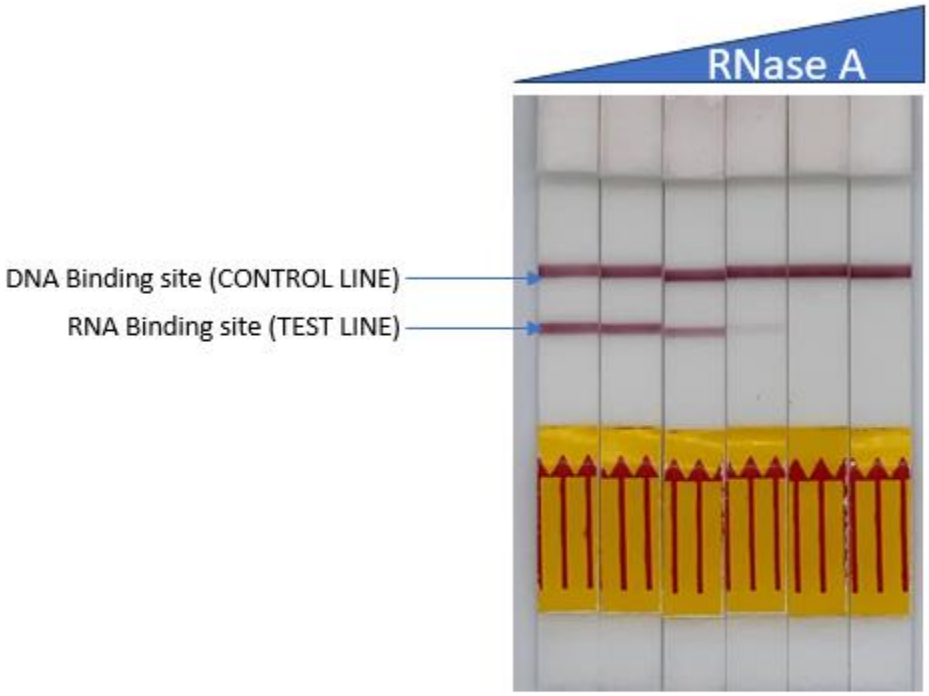
Discussion:

PUBLIC HEALTH RELEVANCE: RNA-based vaccines are a leading tool in the fight against COVID-19 and other diseases. mRNA molecules used to make these vaccines are extremely vulnerable to ribonuclease contaminants during the manufacturing process. Furthermore, additional RNA vaccines and therapeutics are under development. The Attogene RNAseFLOW rapid and portable test to detect ribonucleases in RNA laboratories and manufacturing facilities can greatly assist with the quality control of these efforts.

Importantly, the sequence of the RNA substrate used in our test has been carefully optimized to detect several RNases, including RNase A, RNase T1, RNase I, micrococcal nuclease, S1 nuclease, mung bean nuclease, and Benzonase. Thus, the assay detects all the common classes of RNAses. In addition, the assay is also readily adaptable to any and all nucleases of customer concern. For custom designed testing needs, please contact us at sales@attogene.com.

As all molecular biologists are keenly aware, it is vital that all materials used should be RNase-free when working with RNA. The RNAseFLOW assay contains positive and negative controls for verification and full confidence in the results that are obtained. To perform quantitative assays, the test strips can be added

into standard lateral flow readers to provide numerical readouts (Figure 4). Quantitative records, of course, are critical in daily or weekly quality monitoring programs.



Name	Control	Test	Ratio
0ng RNase A	983574	947045	0.96
30pg RNase A	1174446	717411	0.61
125pg RNase A	1177287	550481	0.47
250pg RNase A	1265830	124128	0.1
500pg RNase A	1368315	8469	0.01
1,000 RNase A	1350055	5000	0

Figure 4. Evaluation of RNase A titration using RNaseFlOW followed by reading in a lateral flow reader. Seven wells were prepared with RNaseFlOW reaction buffer with the indicated amounts of RNase A added. Next, the 2X RNaseFlOW running buffer was added, mixed, and strips were placed in each well. 15 minutes later the strips were removed and analyzed in a lateral flow reader. Numerical results from the reader are shown below with control, test line and ratio.