Development of dsRNA Detection by Dot Blot Technique

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Background

The increasing importance of in vitro transcribed (IVT) mRNA for synthesizing the encoded therapeutic proteins in vivo (as therapeutic proteins or vaccines) demands the manufacturing of pure messenger RNA (mRNA) products. mRNA is synthesized through a four-step process: linear plasmid production, in vitro transcription (IVT), purification and final quality control. It is imperative that the manufacturing process is closely regulated, and manufacturers must meticulously monitor critical quality attributes. This ensures consistency across lot-to-lot production and proof of purity. It also guarantees control over process and product related impurities maintaining them at levels within the acceptable limits as per regulatory guidelines.[2]

Double stranded ribonucleic acid (dsRNA) is a major product-related impurity found in mRNA therapeutics resulting from the in vitro transcription step (Figure 1).[1] Removal of dsRNA remains a manufacturing challenge, as dsRNA shares similar size and intrinsic characteristics with the desired mRNA. While the downstream purification process steps are capable of removing aberrant mRNA species including dsRNA, any lapse in the purification steps can lead to a mRNA product with reduced translational efficiency and undesirable immune-stimulatory profile.

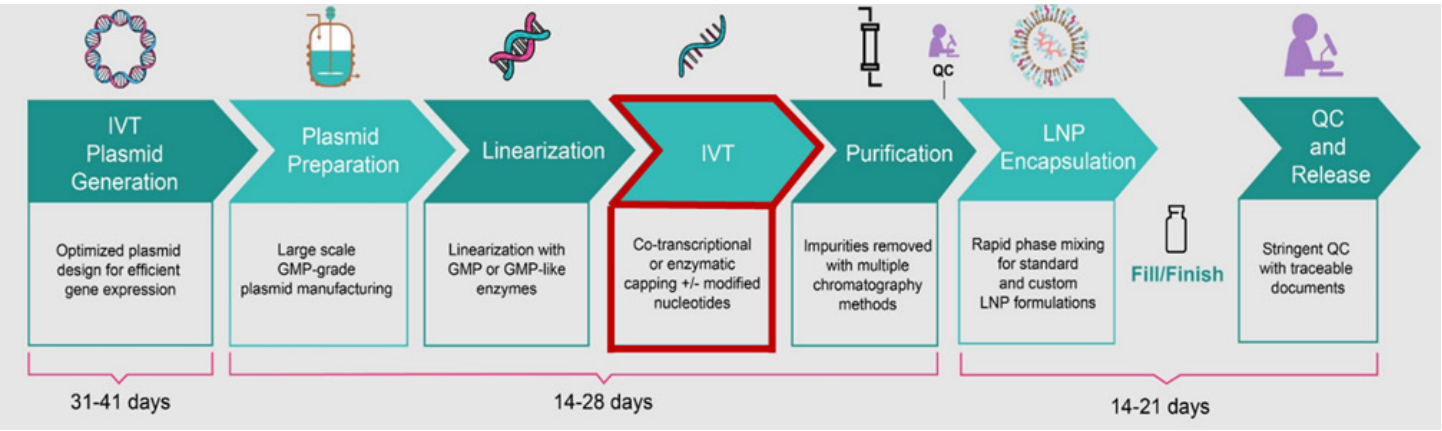

Figure 1: Workflow for manufacturing of mRNA and generation of dsRNA impurities.

Detection of dsRNA by Dot Blot

Principle: The RNA dot blot represents a simplified version of the northern blot enabling rapid and specific identification of RNA molecules of interest. Samples are spotted onto the membrane and subjected to incubation with a primary antibody specific to the target dsRNA. Subsequent to this, another incubation step facilitates the specific binding of horseradish peroxidase (HRP) conjugated secondary antibody to the primary antibody. Upon binding, the HRP conjugate exhibits luminescence through interaction with an enhanced chemiluminescent substrate. The luminescence produced by each dot is directly proportional to concentration of the dsRNA substrate (Figure 2).[3,4]

Method: BA Sciences sought to develop a flexible method for the detection of dsRNA utilizing dot blot technique with imaging and analysis performed on a chemiluminescent imaging system. The method uses a 142 bp dsRNA positive control as a reference standard and demonstrates specificity by use of a single stranded RNA (ssRNA) negative control. The primary antibody used for this assay is mouse anti-dsRNA antibody (J2), which recognizes dsRNA provided that the length of the helix is ≥40 bp. The J2 anti-dsRNA IgG2a monoclonal antibody has been the gold standard in dsRNA detection for the past decade.

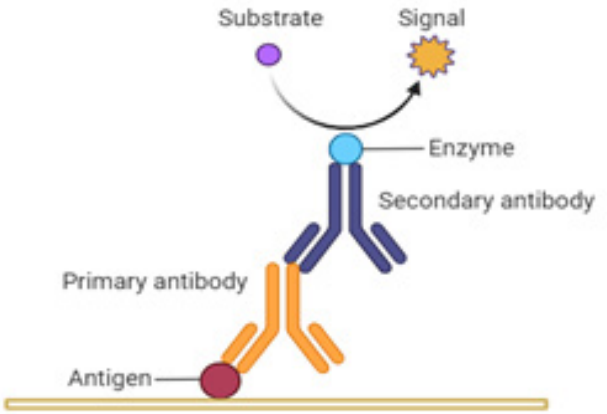

Figure 2: Simplified diagram of the dot blot antibody binding mechanism

This assay offers a qualitative analysis of dsRNA contamination in mRNA samples using a positive control dsRNA gradient. The lowest concentration of dsRNA detectable (LOD) on the membrane was shown to be 3.9 ng (Figure 3, Lanes 1, 2 and 3). In addition to LOD, the assay demonstrates specific recognition of dsRNA, as sample blots containing ssRNA do not appear on the membrane (Figure 3, Lane 5).

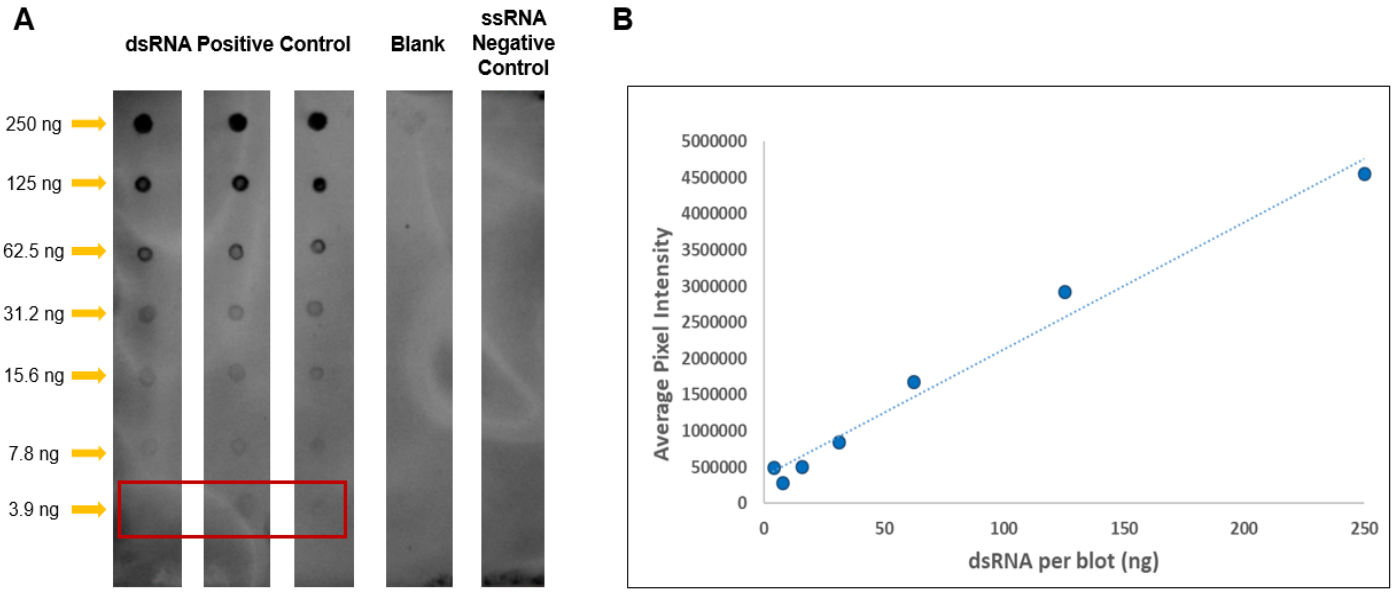

Figure 3: Dot blot demonstrating specificity and LOD of the method. An LOD of 3.9 ng was observed for dsRNA (A). A direct correlation between ng of dsRNA and pixel intensity was established (B).

Conclusion and Future Directions

BA Sciences has developed an efficient and target specific method for the detection of dsRNA contaminants in therapeutic mRNA products. The assay has been demonstrated to be specific towards dsRNA when blotted alongside ssRNA negative controls. Presently, the assay can detect a minimum of 3.9 ng of dsRNA. In addition to the current dot blot method, BA Sciences is working towards developing an ELISA based method for quantitation of dsRNA impurities in mRNA products.

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