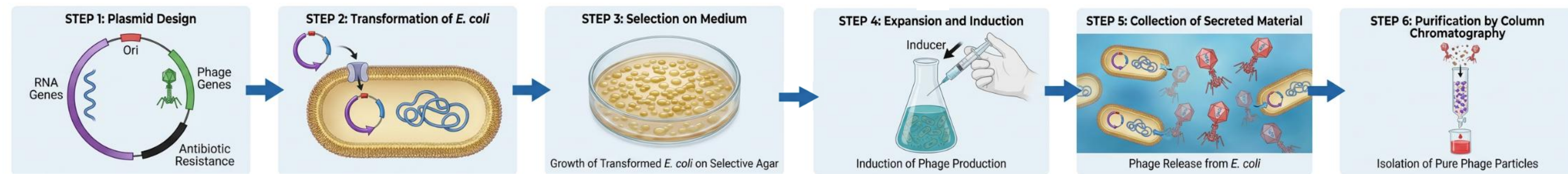


## BACKGROUND

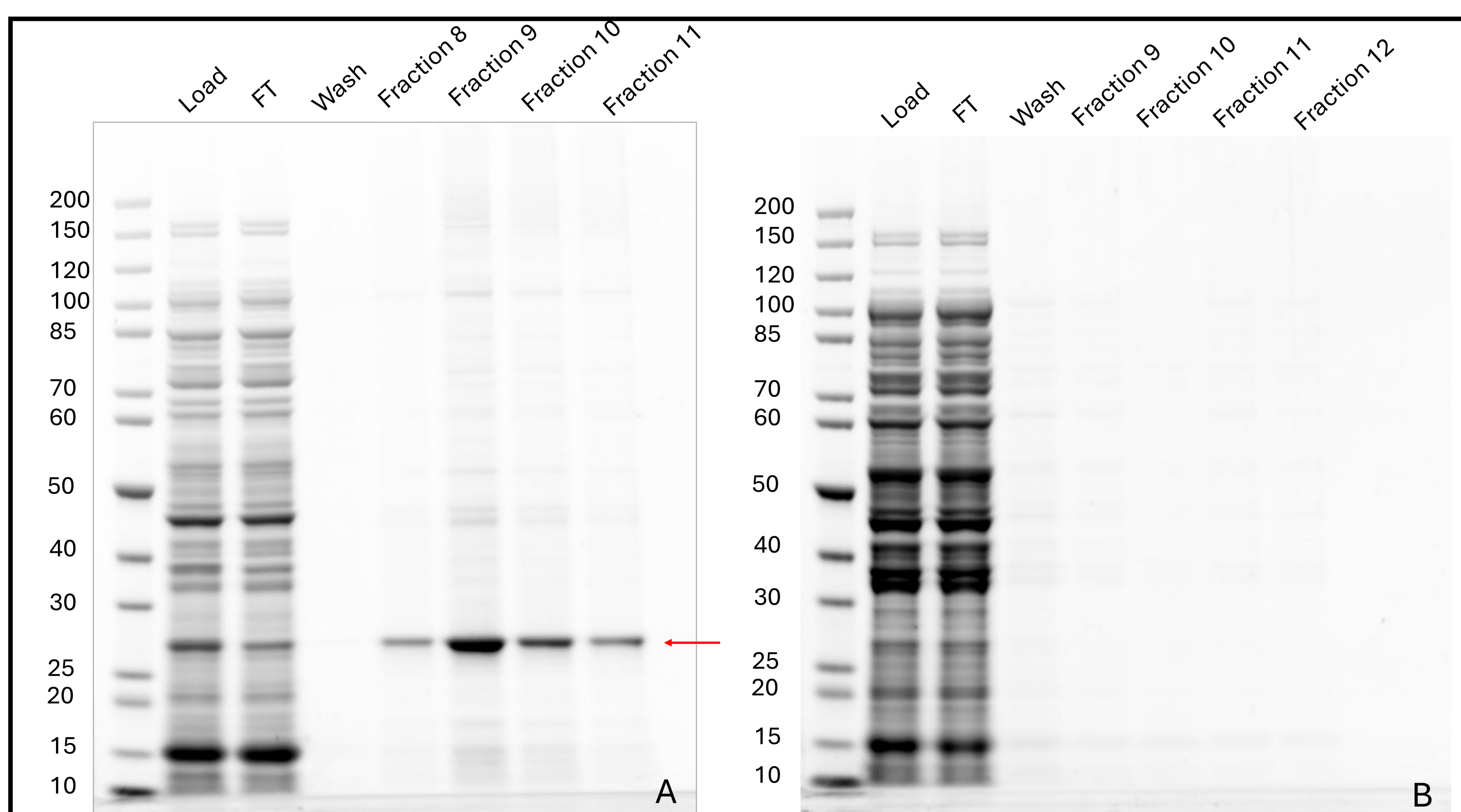
PCR diagnostics for outbreaks of emerging RNA pathogens require controls that verify the full workflow from lysis/extraction through reverse transcription to amplification, yet such outbreaks are marked by lack of accessible native virus and biosafety constraints. Conventional naked RNA controls are RNase-sensitive and can bypass extraction, limiting detection of upstream failures, while whole-virus controls improve commutability but pose biosafety and manufacturing hurdles. We engineered a non-infectious, phage-encapsulated RNA control that protects the target RNA and mimics viral RNA behavior during processing. Configurable as an internal or external control, it provides a robust end-to-end tool for assay development, troubleshooting, and validation with demonstrable commercial IVD utility especially during outbreaks of emerging viral pathogens.

## METHODS

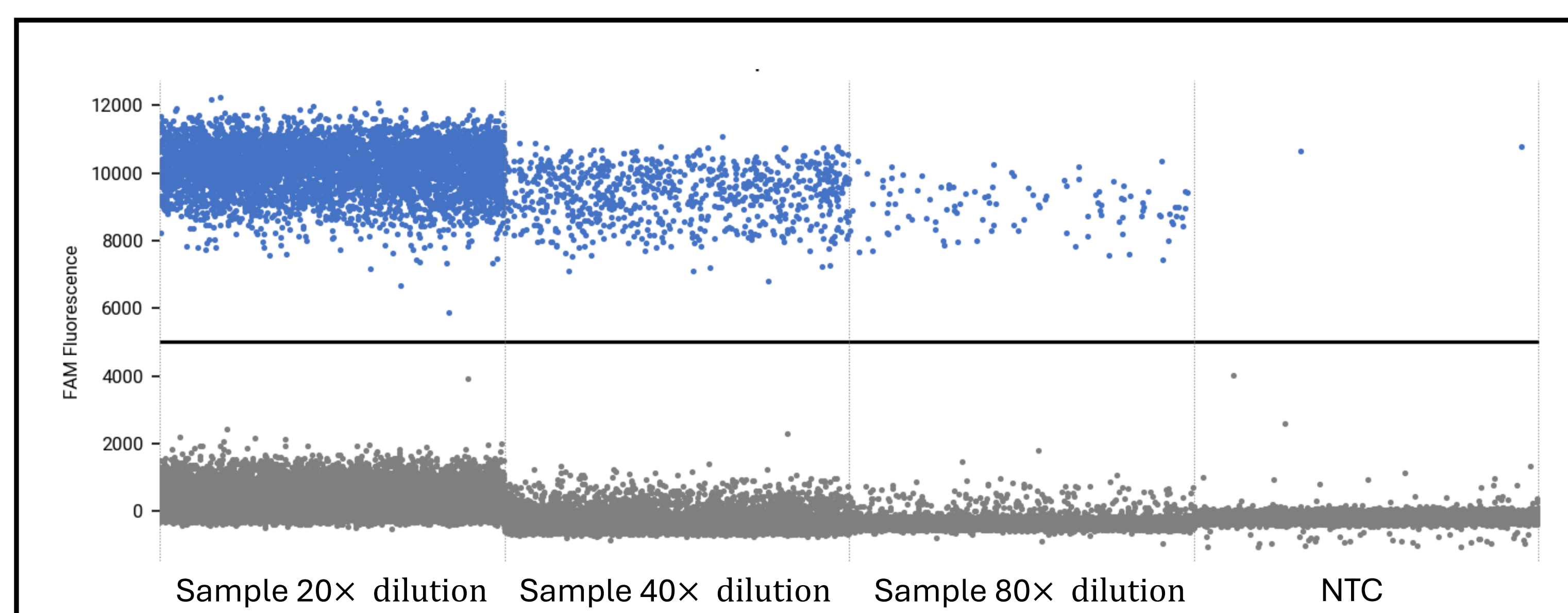
### RNA-ENCAPSULATED PHAGE GENERATION WORKFLOW



## RESULTS

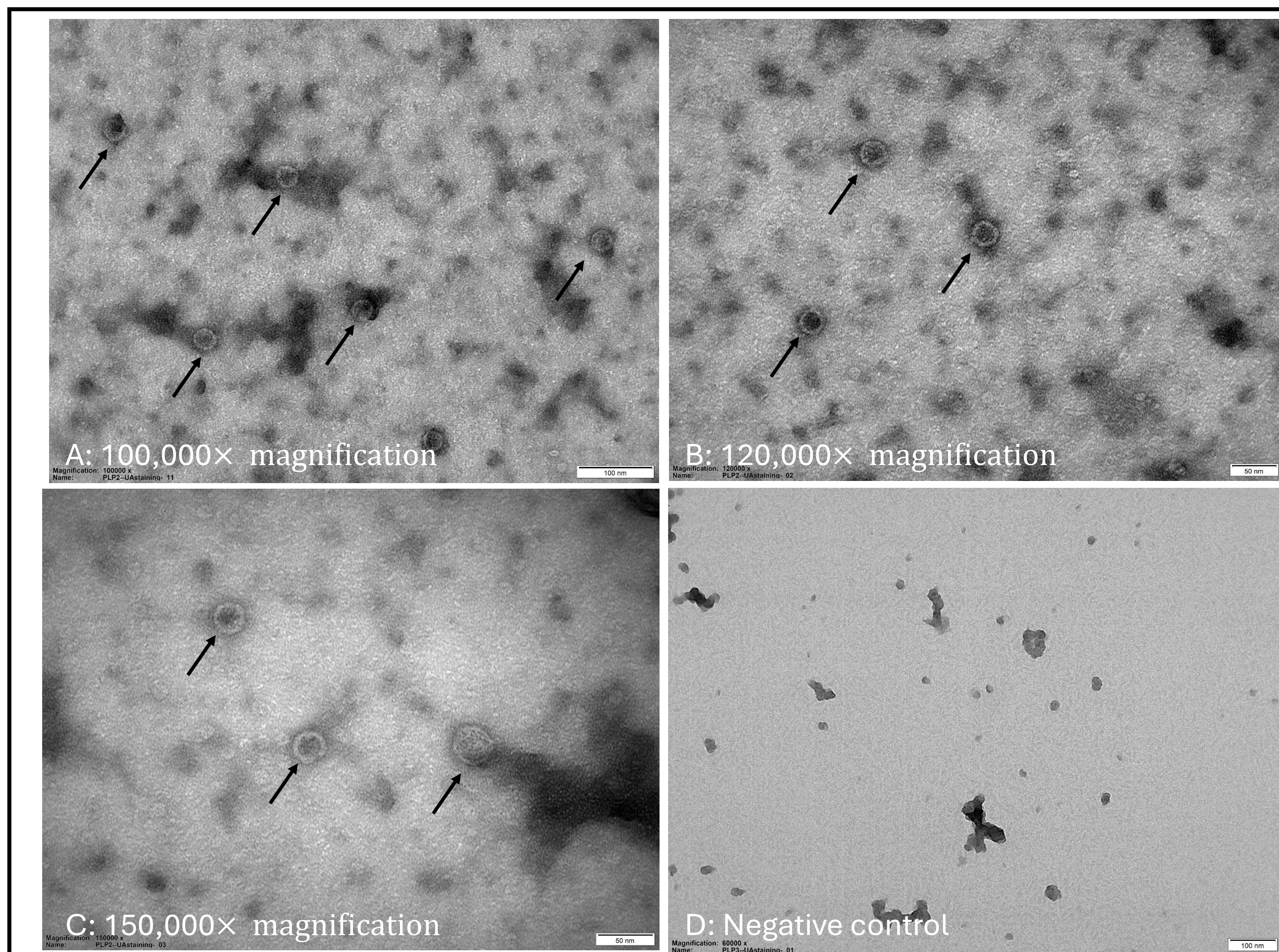


**Figure 1.** SDS-PAGE of expressed phage protein required for phage self-assembly. A) Induced: proteins are expressed, B) Non-induced control: no expression.



**Figure 3.** Titration and quantification of purified phage-protected RNA by digital reverse transcription PCR (dRT PCR).

**Concentration of HCV RNA control as determined by dRT PCR**  
 $1.6 \times 10^9$  copies/mL



**Figure 2.** A-C) Electron Microscopy of 27 nm purified phage containing protected RNA at different magnifications; D) Negative control.

### Resistance of Phage-protected RNA to digestion by nuclease

Assay	After treatment I:	After treatment II:
	Benzonase treatment: 3h, 37°C	Benzonase treatment A: 3h, 37°C Benzonase treatment B: 16h, 4°C
HCV dRT PCR	$1.6 \times 10^9$ copies/mL	$1.2 \times 10^9$ copies/mL

**Table 1.** dRT PCR results of Phage-protected RNA treated with nuclease (benzonase).

### Compatibility of Phage-protected RNA material with diagnostic assay

Assay	Assay Version	Assay Type
Xpert HCV VL Fingerstick	1	In Vitro Diagnostic

Test Result: HCV DETECTED > 1.00E08 IU/mL (log 8.00)

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
HCV	16.3	577	POS	PASS
IQS-H	23.9	222	PASS	PASS
IQS-L	33.0	516	PASS	PASS

**Table 2.** Performance of phage-protected RNA in Xpert HCV assay (10x dilution).

## CONCLUSIONS

Using molecular biology techniques, we successfully generated a modified self-assembled particle containing a protected RNA target. The product can be produced at scale and purified efficiently using chromatography, yielding a highly purified preparation with robust RNA protection. RNA encapsulated in this manner is resistant to nuclease degradation, making it well suited for use as stable assay controls. Importantly, the particle-based RNA control was validated in an approved diagnostic assay and performed as expected, demonstrating assay compatibility and confirming the validity of this design strategy. This platform provides a rapid, scalable method for generating RNA assay controls particularly in situations requiring rapid deployment, such as public health emergencies or the emergence of novel pathogens.

## CONTACT

Pavel Zhelev, Director, Product Management; Email: pavel.zhelev@microbix.com