

Towards the agnostic future of diagnostics: untargeted nanopore sequencing of biological fluids

Adina Doyle^{1,2}, Pierce Roth^{1,3}, Alvin Liem^{1,3}, Jessica Hill^{1,3}, Samir Deshpande¹, Jennifer Lee^{1,4} and Maria Arévalo¹

¹U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD; ²Excet, Springfield, VA; ³DCS Inc, Belcamp, MD

⁴DTRA, Ft. Belvoir, VA

ABSTRACT

Current methods for diagnosis of bacterial and viral infections include cultivation of microorganisms, identification of antigens, detection of antibodies, and detection of nucleic acids. These are all targeted approaches, and new assays must be developed and implemented for new threats in lengthy development and approval processes. Therefore, development of agnostic platforms will be crucial to avoid delays in diagnostics, treatment, and development of countermeasures. Nanopore sequencing technologies are quickly evolving and provide low-cost, portable, easy-to-set up devices to rapidly detect biological threats without the limitations of targeted approaches or target panels. Long reads and live base calling coupled with appropriate bioinformatics tools allows entire genomes to be analyzed even in complex sample matrices for detection, even in the case of previously unknown human pathogens or engineered threats. Our overall scientific goal is to establish and validate sequencing protocols to detect human pathogens from biological fluids that are routinely used for disease diagnosis, can be easily collected by clinicians, and are minimally invasive: saliva, urine, and blood. A full process from sample collection, stabilization and storage, processing, untargeted nanopore sequencing, and bioinformatic methods will be developed for the identification of bacterial and viral pathogens from these biological fluids. Current work is focused on workflows towards untargeted identification of bloodborne pathogens. Successful methods to store samples (under different temperature conditions) and recover microbial nucleic acids are being earmarked. Sequencing conditions and bioinformatic pipelines are continuously being assessed and optimized to generate results that are clear and inspire confidence in making a timely diagnosis. Successful implementation of an untargeted nanopore sequencing system for diagnostics would enable rapid identification of any new, unknown, or engineered microorganism at even remote or low-resource sites of outbreaks to inform decision making.

TECHNICAL APPROACH

1. Develop sample collection, stabilization/storage, processing, untargeted nanopore sequencing, and bioinformatic identification methods for bloodborne pathogens including bacteria, DNA viruses, and RNA viruses. Assays will be developed for blood products: whole blood, plasma, and serum.
2. Develop processes for pathogens that are shed in urine.
3. Develop processes for respiratory pathogens that can be found in saliva.
4. Simplify/reduce sample preparation time and streamline the three processes and consumables into a single system for agnostic detection.
5. Option years: Develop automated sample preparation system.

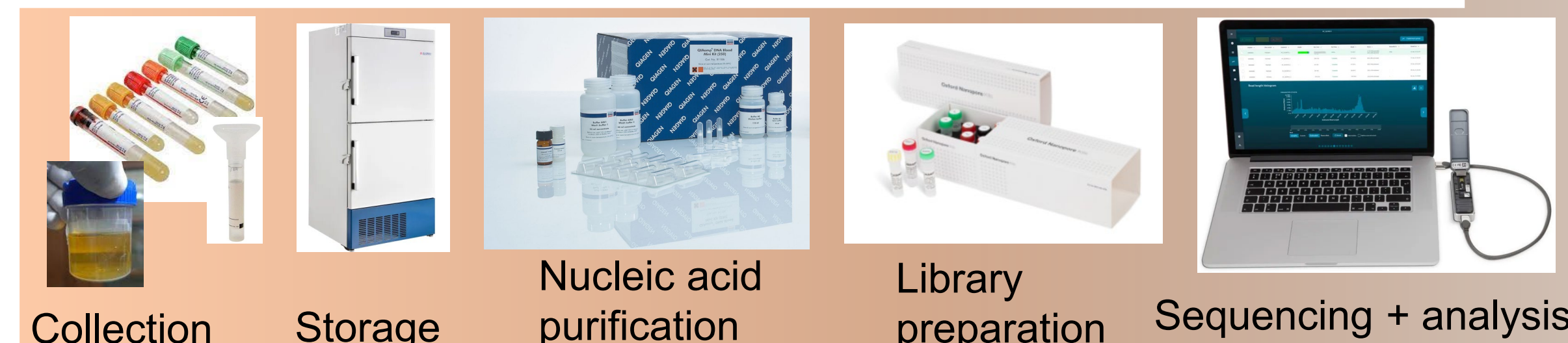


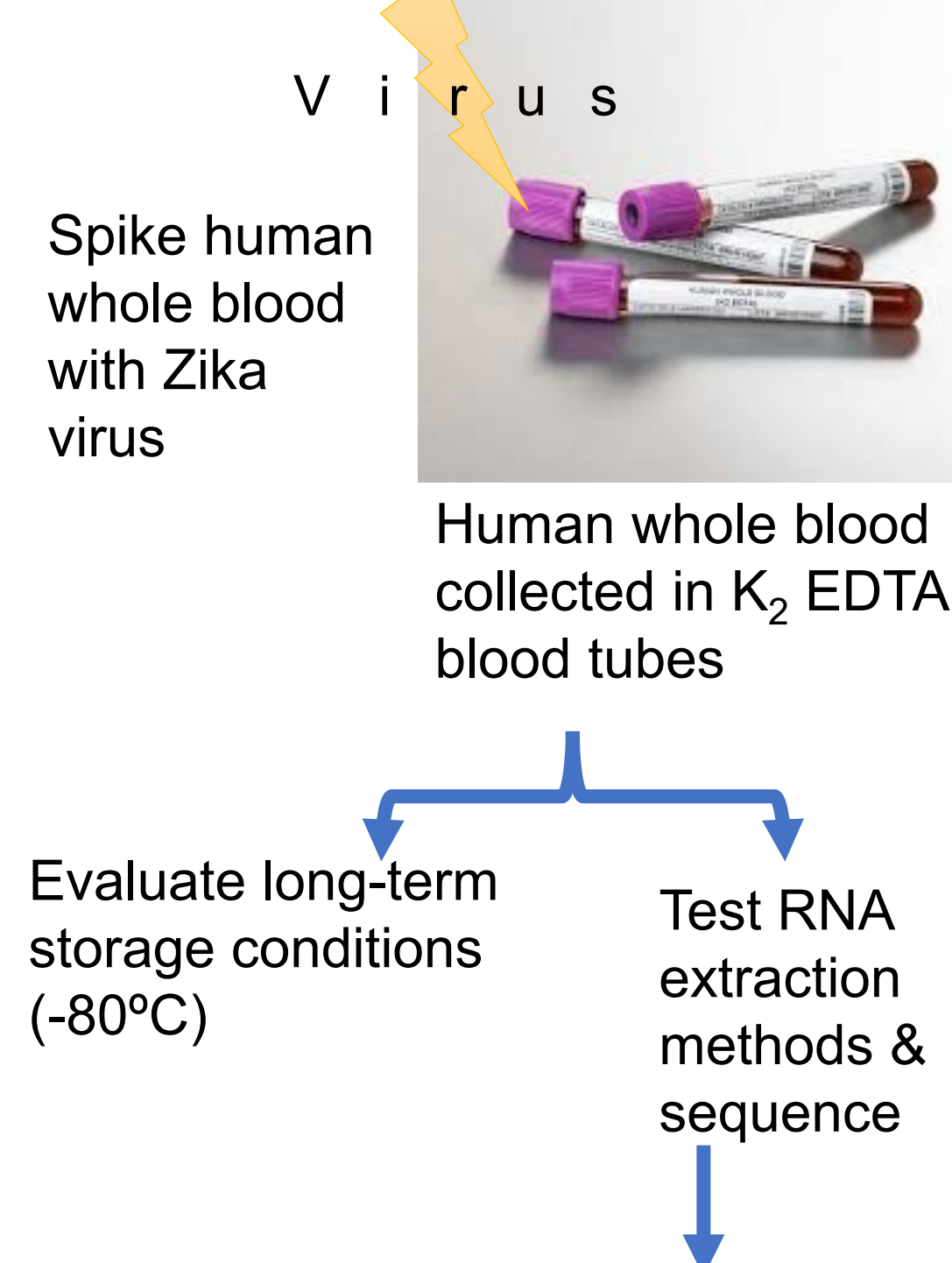
Fig. 1. Developing diagnostic process from sample collection to result



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Fig. 2. Initial Workflow for Evaluating RNA extraction kits for blood



Initial/Standard Workflow

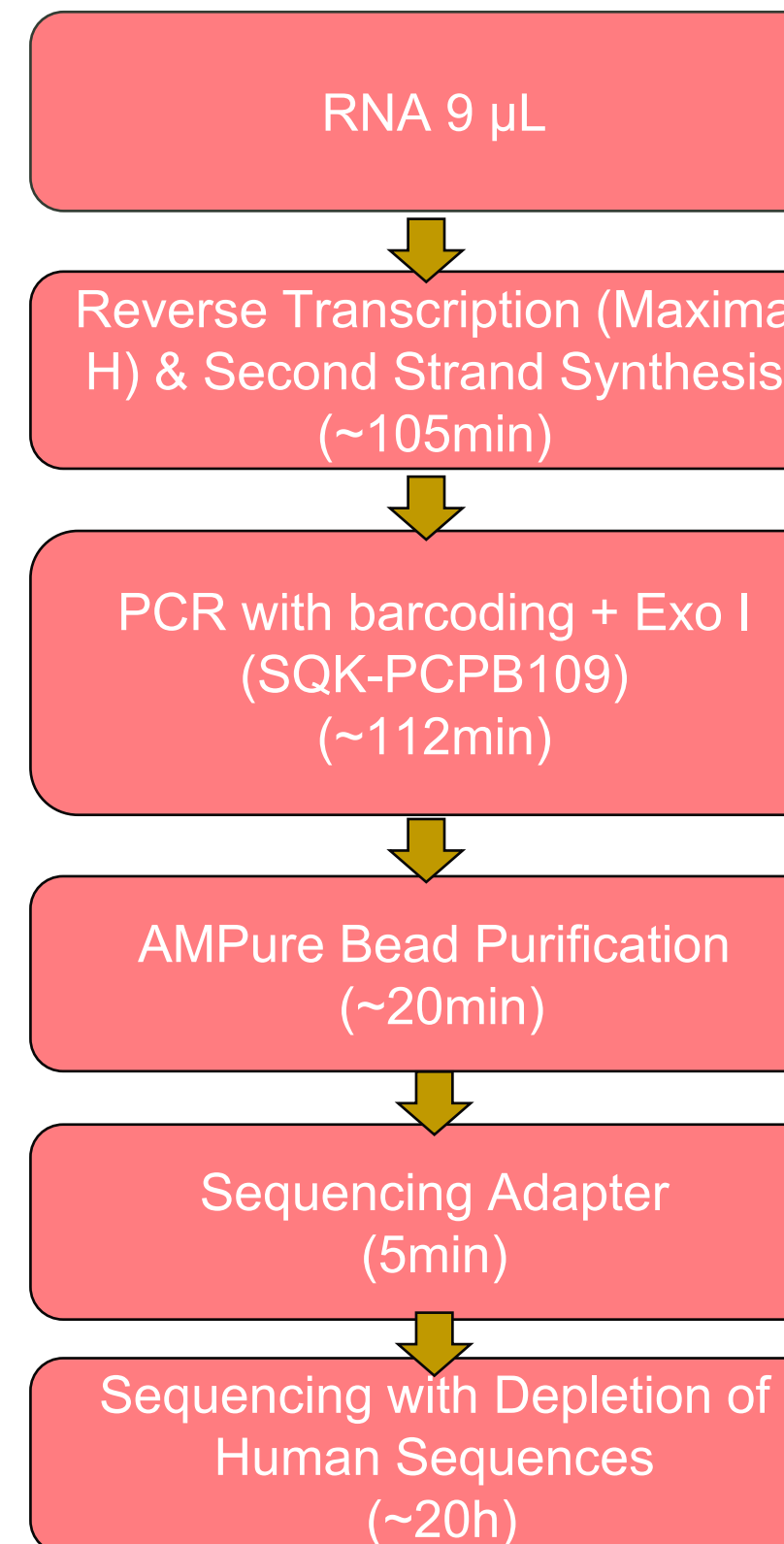


Fig. 3. Comparison of Zymo Quick RNA Whole Blood Vs. QIAamp RNA Blood Mini Extraction on Identification of Zika Virus in Spiked Whole Blood

Results shown were analyzed using Centrifuge. The number of reads mapping to the Zika virus genome are reported by viral input.

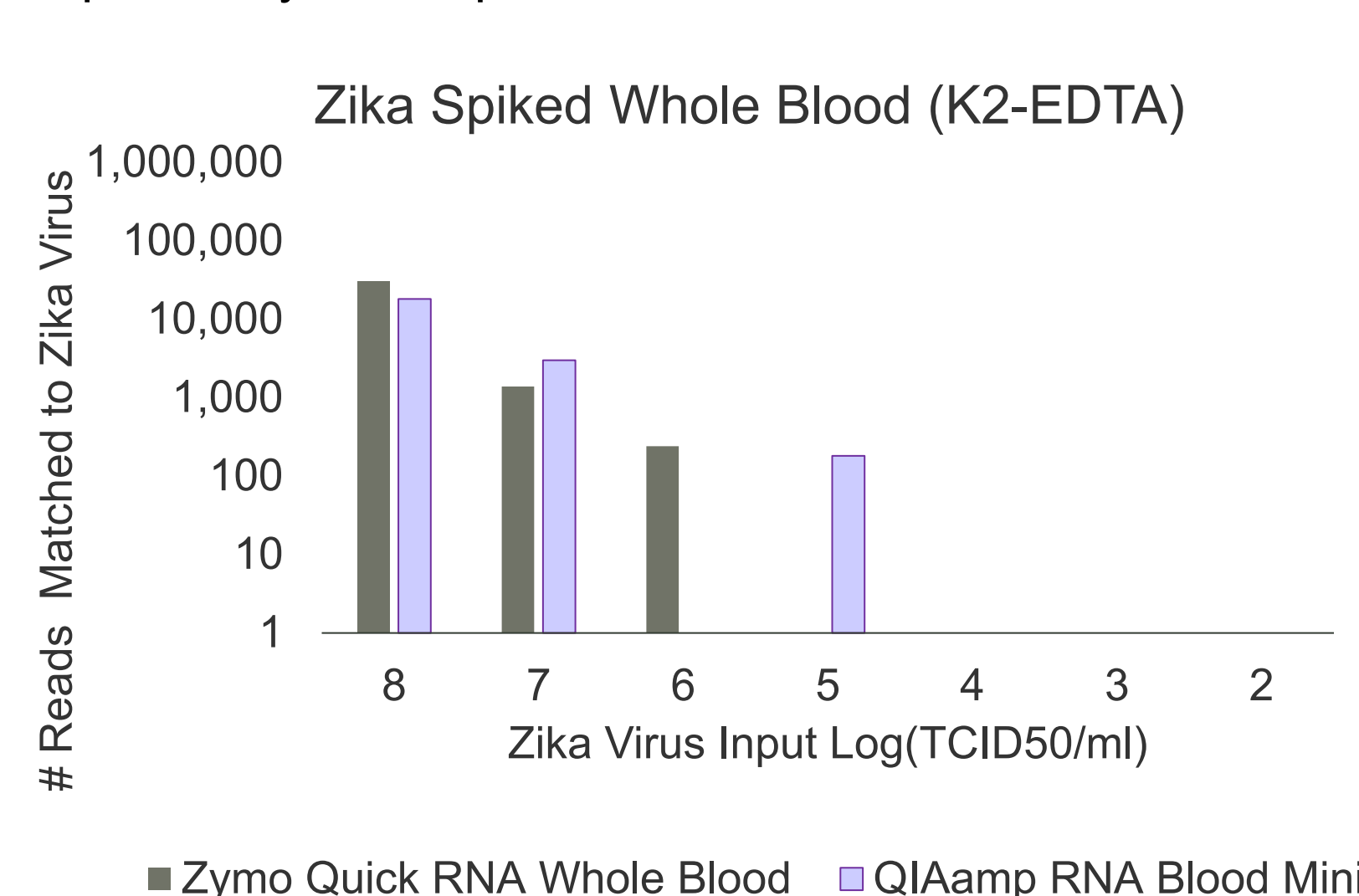


Fig. 4. Comparison of Anti-Coagulants Used in Blood Collection Tubes on Sequencing

Zika virus was spiked into blood that was previously collected in standard anti-coagulants: Na Heparin (green tops), K₂ EDTA (purple/lavender tops), or Na EDTA (purple/lavender tops). Results shown were analyzed using Centrifuge.

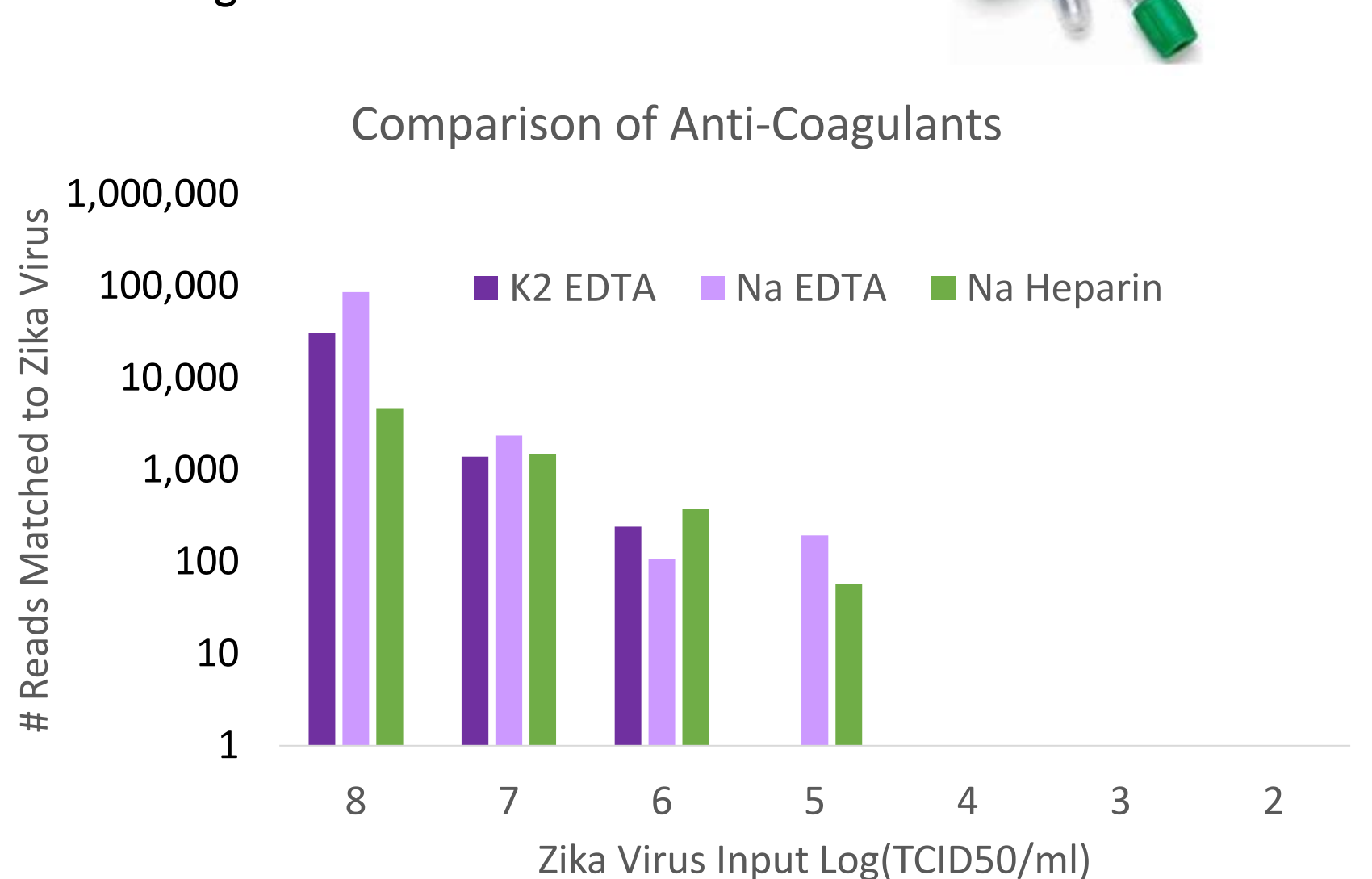


Fig. 5. Improving Library Preparation for Detection of RNA Viruses in Blood.

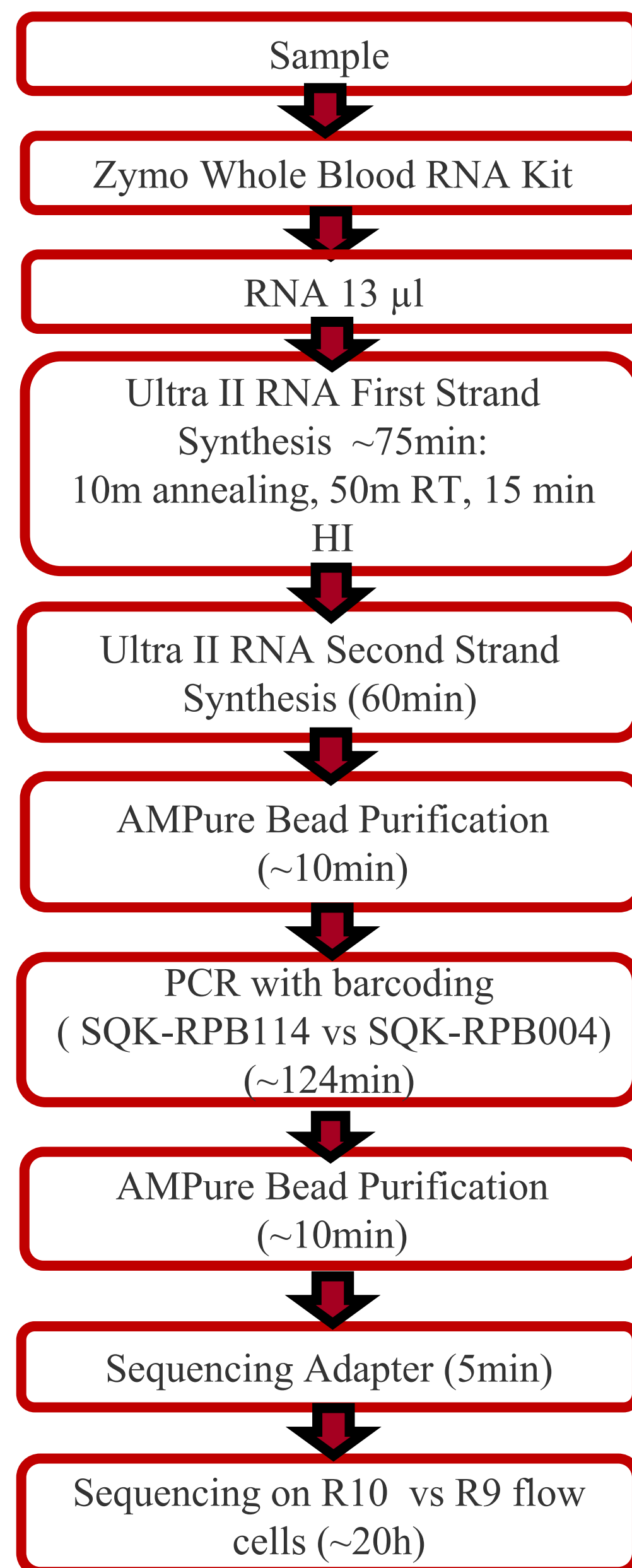


Fig. 6. New Library Preparation Process Improves Assay Sensitivity at Lower Viral Concentrations.

Results were analyzed using Centrifuge.

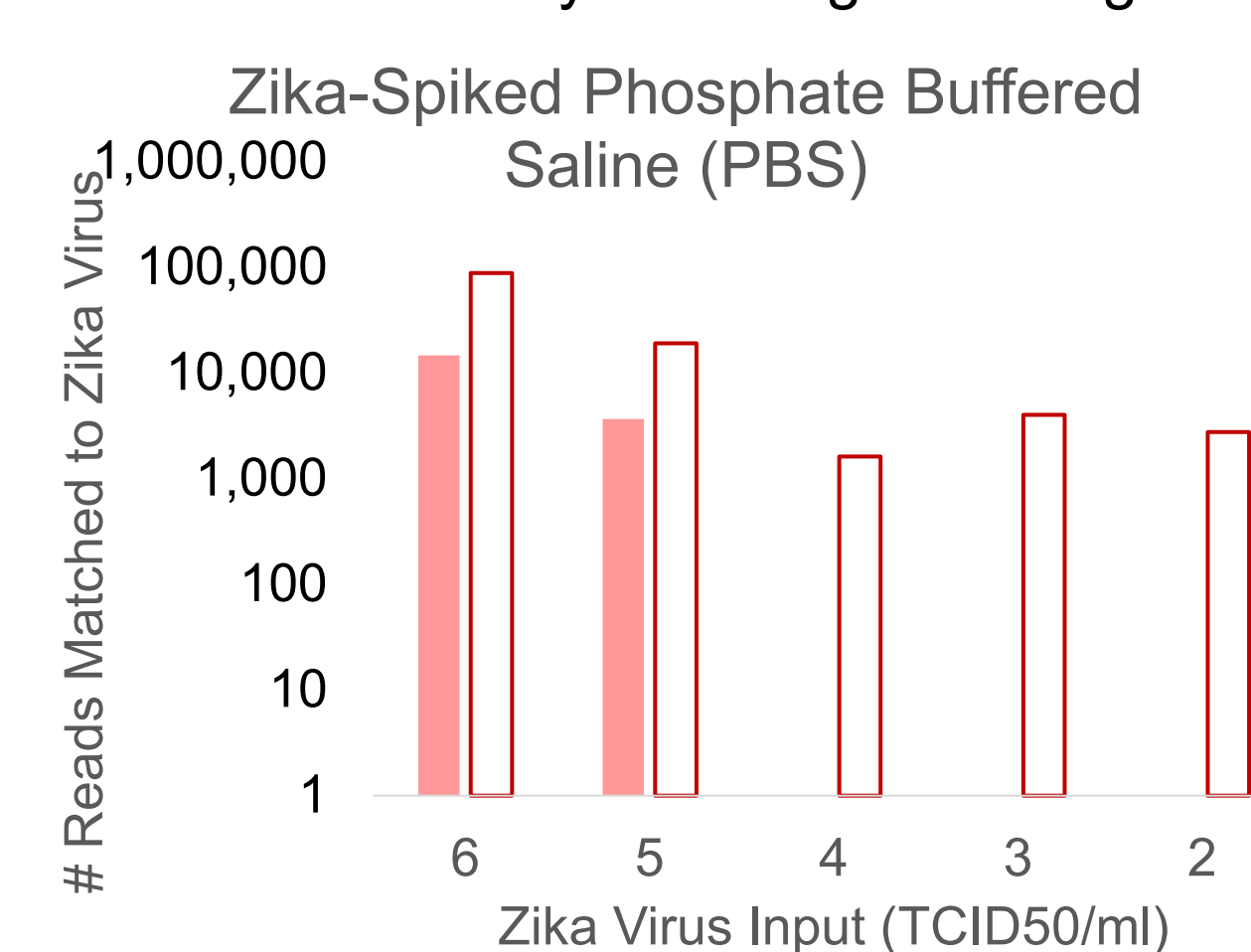


Fig. 7. Adaptation & Comparison to R10 Flow Cells & Associated Kit 14 Reagents. The R10 vs R9 chemistries were compared across different spiked media: A) phosphate buffered saline (PBS), B) whole blood, and C) plasma. Adaptive sequencing was using during acquisition of sequencing data to deplete human sequences. Analyses were performed using Minimap2 with a NCBI RefSeq database truncated to contain viruses only (RefSeq – Viral)

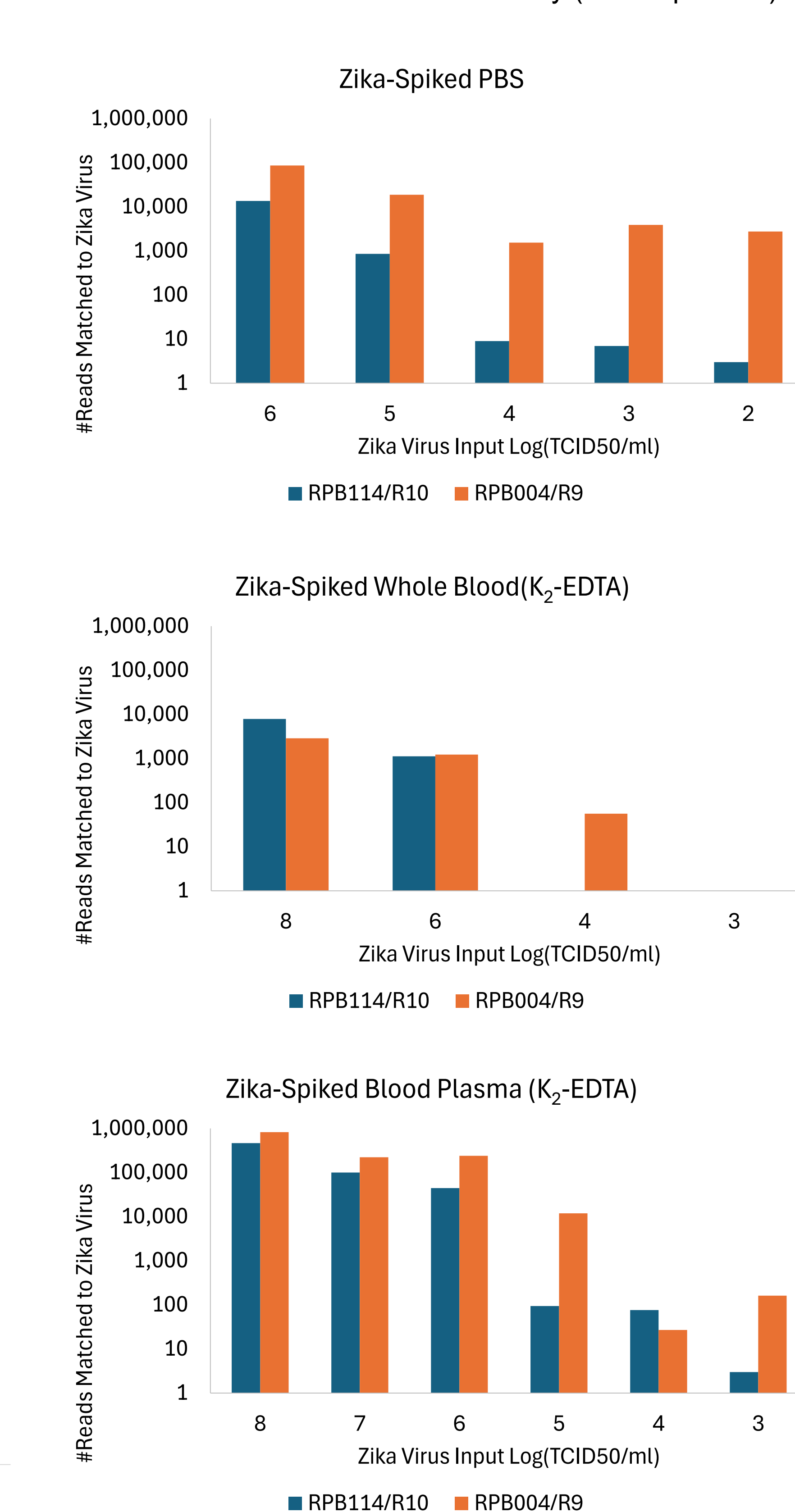
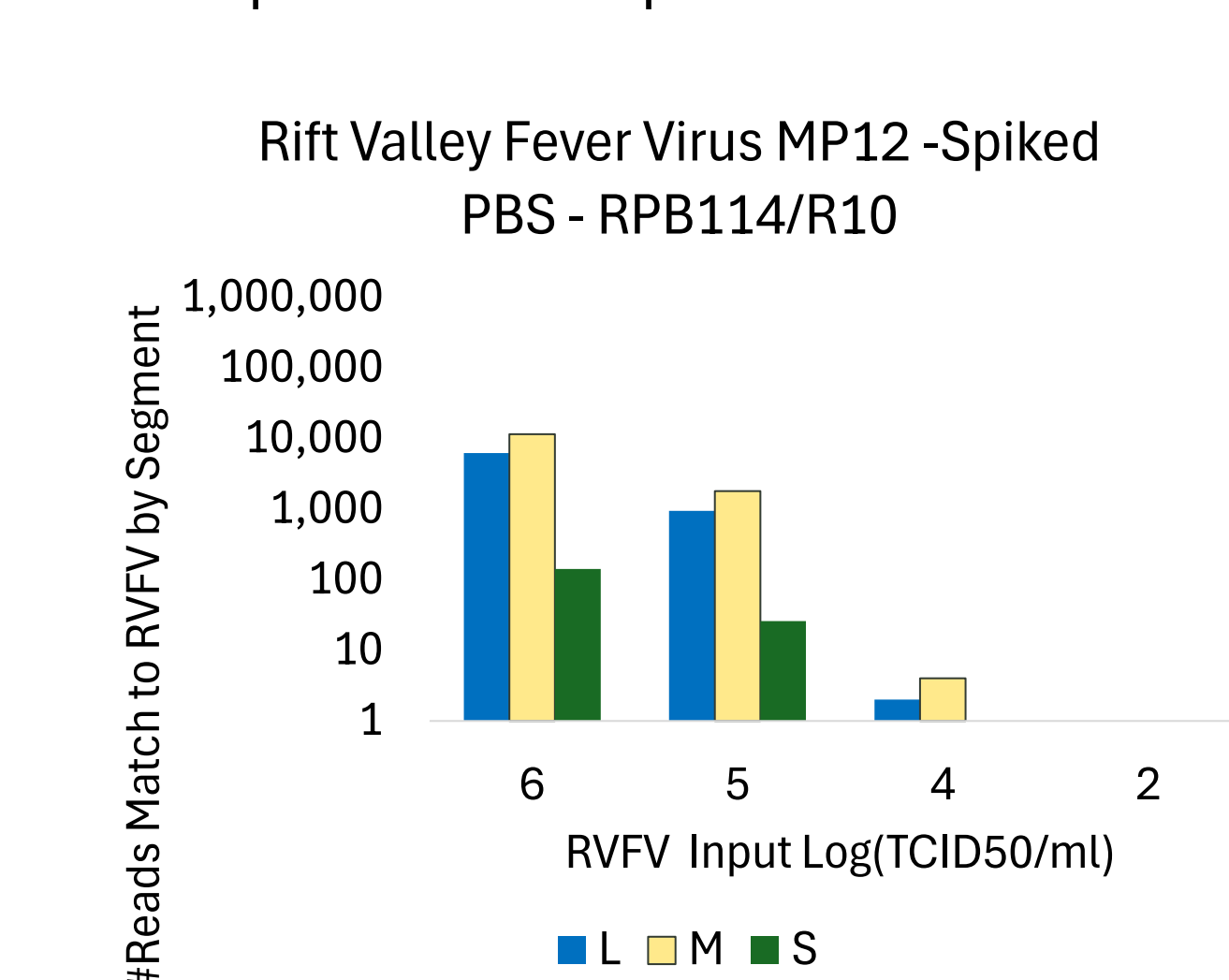


Fig. 8. Detection of Segmented RNA Viruses. Rift Valley fever virus (RVFV) MP12 strain was used to test if our workflow was able to detect segmented viruses. RVFV contains 3 segments designated as L, M, and S. L and M are negative-sense RNA segments while S is an ambisense segment. RVFV was diluted in PBS. Analyses were performed using Minimap2 with RefSeq – Viral database.



SUMMARY

- We developed an untargeted method to detect RNA viruses in human blood.
- The method works for positive-sense, negative-sense, and ambisense genomes.
- Optimization is needed to increase sensitivity.
- Methods for DNA viruses and bacteria are under development.
- Methods for detection of respiratory pathogens from saliva are also under development.

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