LABCORP MOLECULAR BIOANALYTICAL SERVICES

Assessment of Alternative DNA Extraction Methods from Microsamples of Common Matrices Collected for Vector Shedding Purposes in Clinical Trials

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Agenda

- Microsampling Environment Overview
- Preliminary Assessment Review
- Conclusions and Future Considerations Discussion
- Q&A

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Microsampling Environment

Microsampling* emerging as alternative sampling method

Non-Clinical context:

- Lower Volume = Safer
  - Allows more collection points as animals are not at risk with volumes sampled.
  - Increase in endpoints from a single collection timepoint
  - Benefits for 3Rs in animal studies (especially small mammals)
    - replacement, **reduction , refinement**

Clinical context:

- Convenience! Devices are easy to use at home. Leads to improved patient compliance
  - Allows more collection points or multiple at a given timepoint
  - Simplifies sampling in remote areas and for critical patients
- Better experience!
  - Less invasive! Less discomfort!
- Cheaper!
  - Often requires less technical collection procedures and trained staff
- Safer!
  - For rare diseases, Clinical Trials are often in neonates/newborn/infants, so available volumes of certain matrices are lower

***Microsamples:** generally considered samples with volumes **≤50µL**

Key Microsampling Challenge: Quality qPCR vector shedding data

- Vector shedding analysis objective:
  - Detect Viral Vector genomes with high sensitivity
  - It is definitely not possible to do this without extraction of DNA!

- The current Labcorp Standard for Blood DNA extraction is automated and has a standard 220 µL volumetric input:

  - **Microsampling Challenge**:
    - Produce quality data from **4x Less Material**

  - **Sensitivity!**
    - Compound Problem:
      - To maintain sensitivity:
        - Lower Elution Volume (Same amount of target in less volume = better sensitivity)
          - **Reduction in elution efficiency – Low Yield**
          - **Less volume for downstream applications – Impact to repeat testing**

  - **Recovery!** Further loss to alternative and/or non-standard collection devices?

https://www.neoteryx.com/volumetrically-accurate-micro-sampling-vams-collection-devices

Current Blood DNA Extraction Methodologies

QIAsymphony or Kingfisher

- Medium-throughput Automated workflow
- Utilises ~220 µL of sample
- Requisite sample input volume:
  - **Pre-dilution of analyte before extraction**
- Elution volume 100 µL; **Too Large?**
  - **Reducible**

Manual column kits

- Low-throughput Manual workflow
- Utilises 100 µL-400 µL of sample
- Requisite sample input volume:
  - **Pre-dilution of analyte before extraction**
- Elution volume 50-200 µL

Phenol Chloroform

- Low-throughput manual workflow
- Utilises ~250 µl of sample
- Requisite sample input volume:
  - **Pre-dilution of analyte before extraction**
- Final resuspension volume can be adjusted

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Our Preliminary Assessments

Samples and Test System

- Focused initially on human blood samples
- Collected ‘bulk’ blood sample from 1 donor (at this stage), into a standard K2-EDTA vacutainer
- qPCR Positive Control Spiking - 2 Kbp linear plasmid
  - Used standard DNA extraction and qPCR methods Validated/characterised at Labcorp
  - Standard blood extraction: Extraction of 200µL of 220µL load
    - **Spiking Target:** 1.2E6 copies in 200 µL → 50,000 copies in qPCR ($100\%_{TR}$)
    - **Empirically:** 1.11E6 copies in 200 µL → 46,357 copies in qPCR ($100\%_{TR}$)
  - qPCR with 5E7 to 50 copies per reaction in a DNA background up to 1µg.
    - Lowest input volume (3µL): still ~600 copies in qPCR ($100\%_{TR}$)
- Analysed samples input volumes ≤50 µl in the different test methods
- Compared recoveries of DNA for:
  - Various Extraction Methods/Parameters
  - Blood load volumes

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Methods Employed

Candidate Method Selection

- Primary Factor:
  - Input Volumes: Capable of Handling ≤50 µL Blood
- Secondary Factors:
  - Runtime and Cost
    - Automation Possible?
  - Novel Approach: See Method B
  - Intended Application: See Method C

Method A

- Silica based membrane purification, with elution in water or low salt buffer
- Suggested Input Volume: 100 µL to <10µL (Tested 3 µL – 50 µL)
- Elution volume 20 – 100 µL
- Nominal Input: Elution Ratio: 1:1 (50:50)
- Potential For Automation

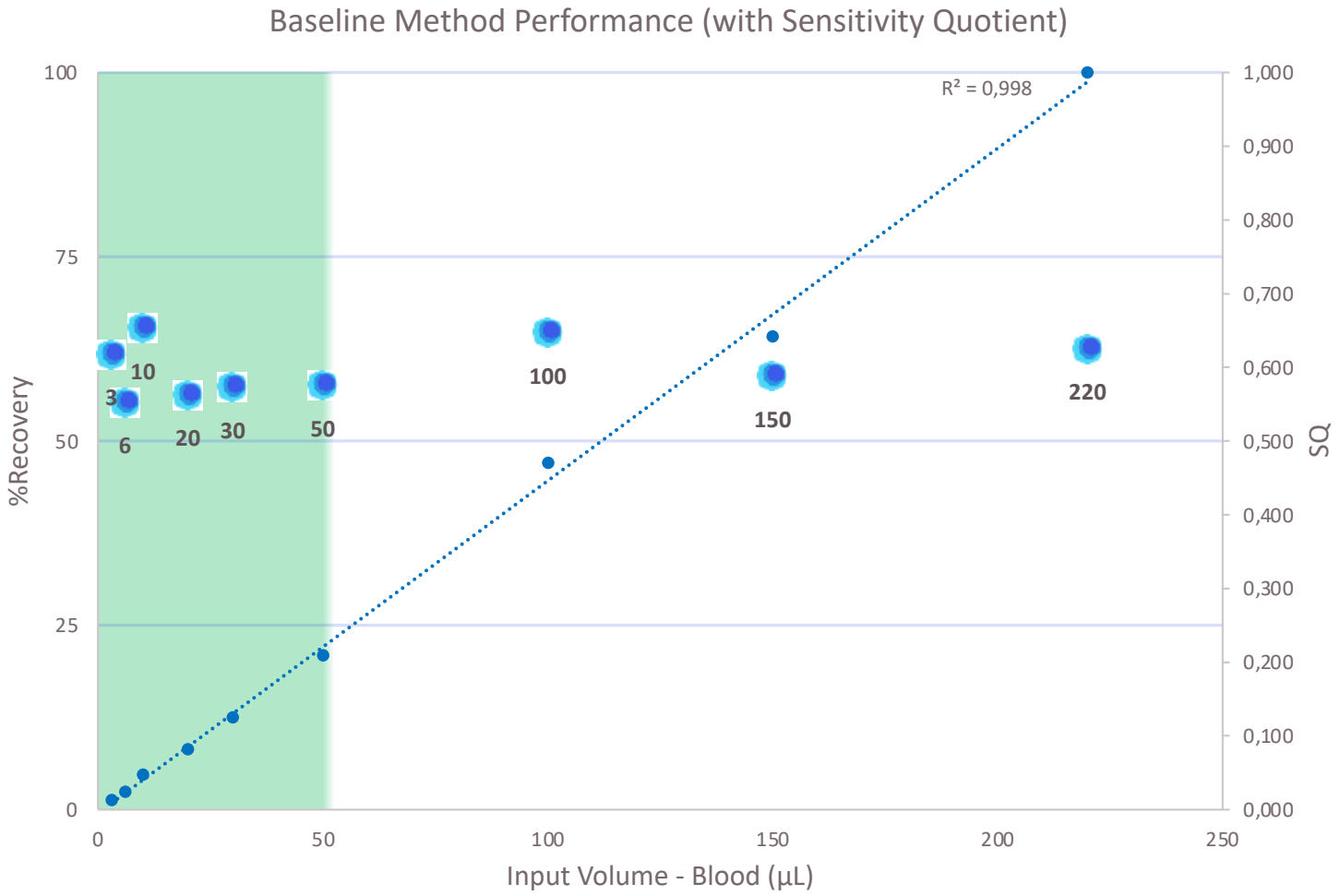
Method B

- Lysis with PCR inhibitor sequestering matrix
- Suggested Input Volume: 3µL to 6 µL range (Tested 3 µL – 20 µL)
- Elution volume ~230 µL
- Nominal Input: Elution Ratio: 1:11.5 (20:230)

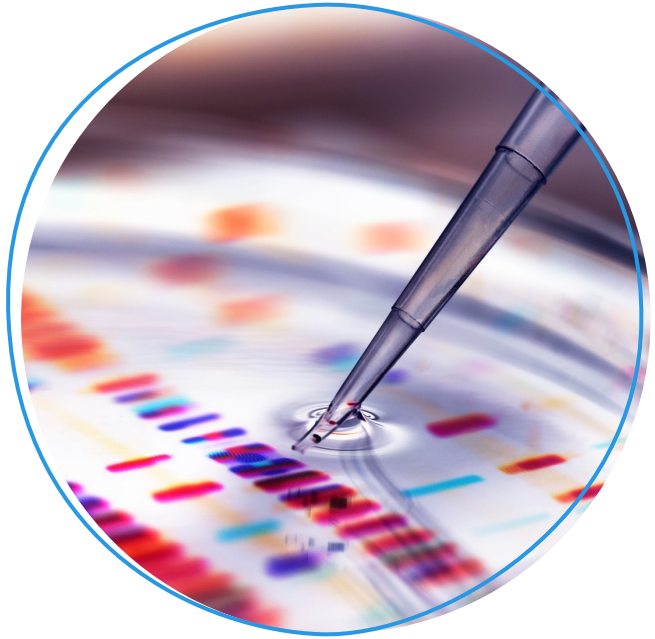
Method C

- Silica based magnetic particle purification, with elution in water or low salt buffer - Marketed Application: Forensics
- Suggested Input Volume: 100 µL to <10µL (Tested 3 µL – 50 µL)
- Elution volume: 100 µL
- Nominal Input: Elution Ratio: 1:2 (50:100)
- Automated

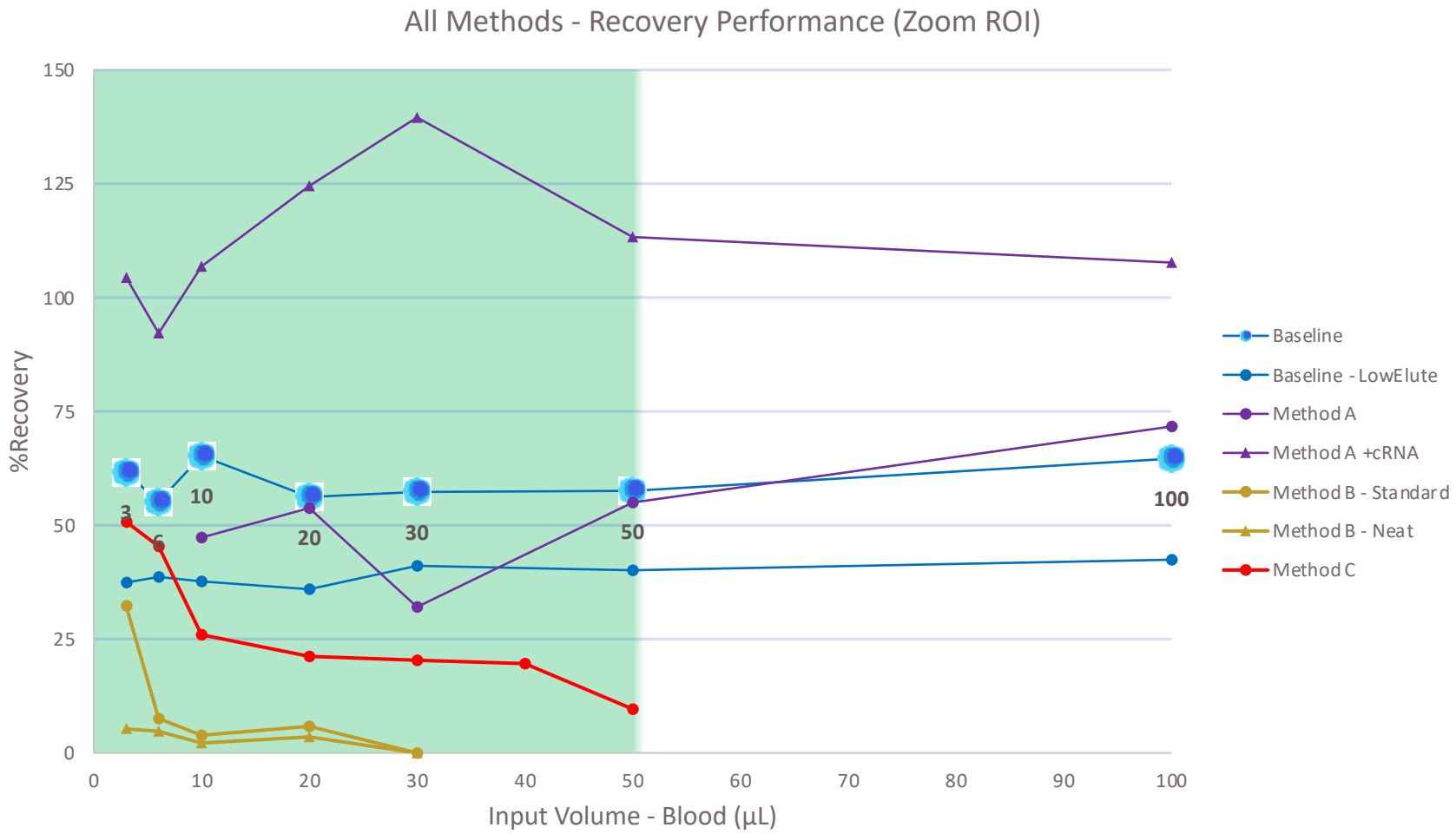
Results – Our Standard Method - Baseline



- Surprisingly:
  - **Conserved Recovery Efficiency across the range Tested**
- However:
  - **Clear attenuation of Sensitivity**

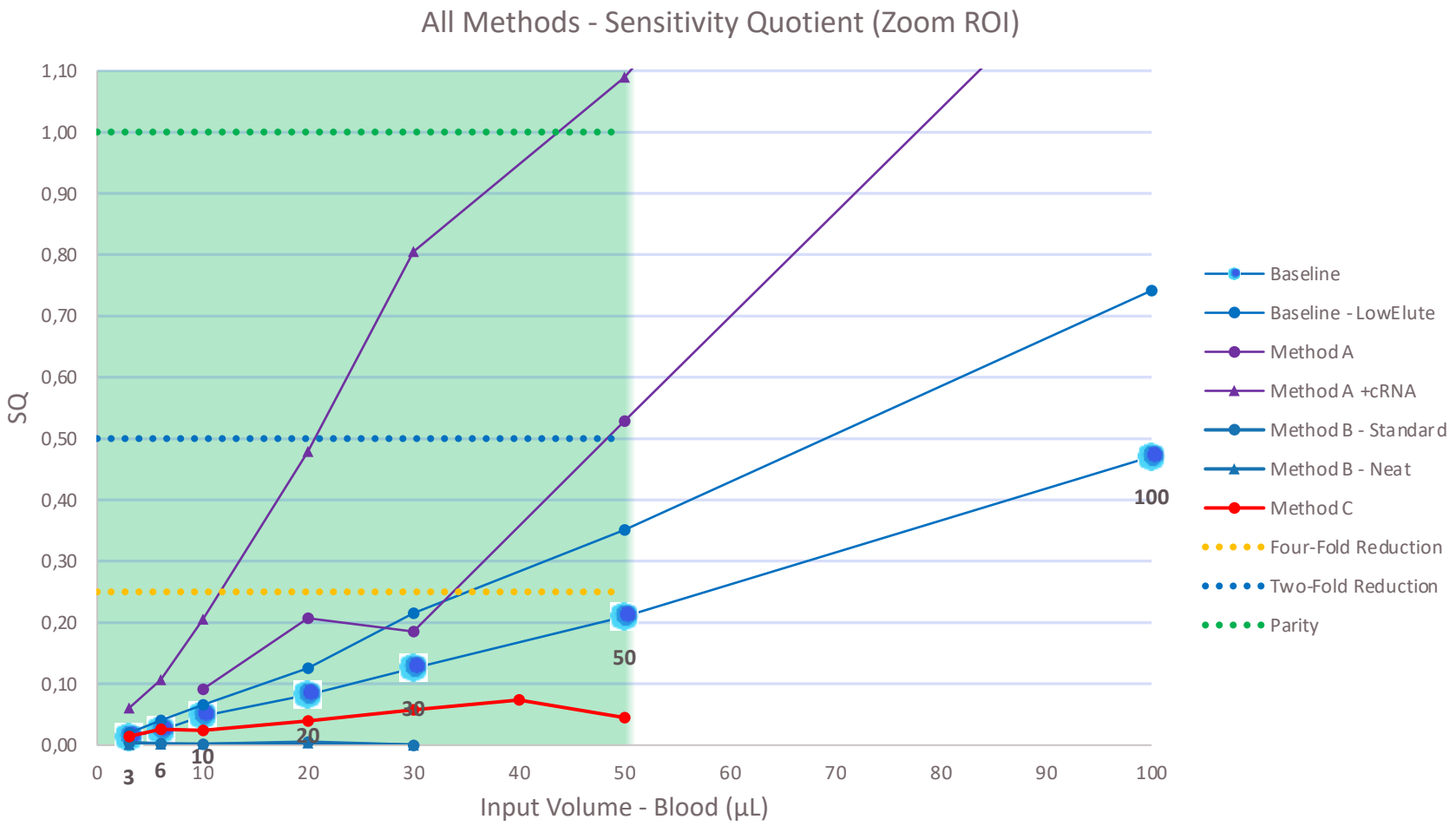


Results – All methods



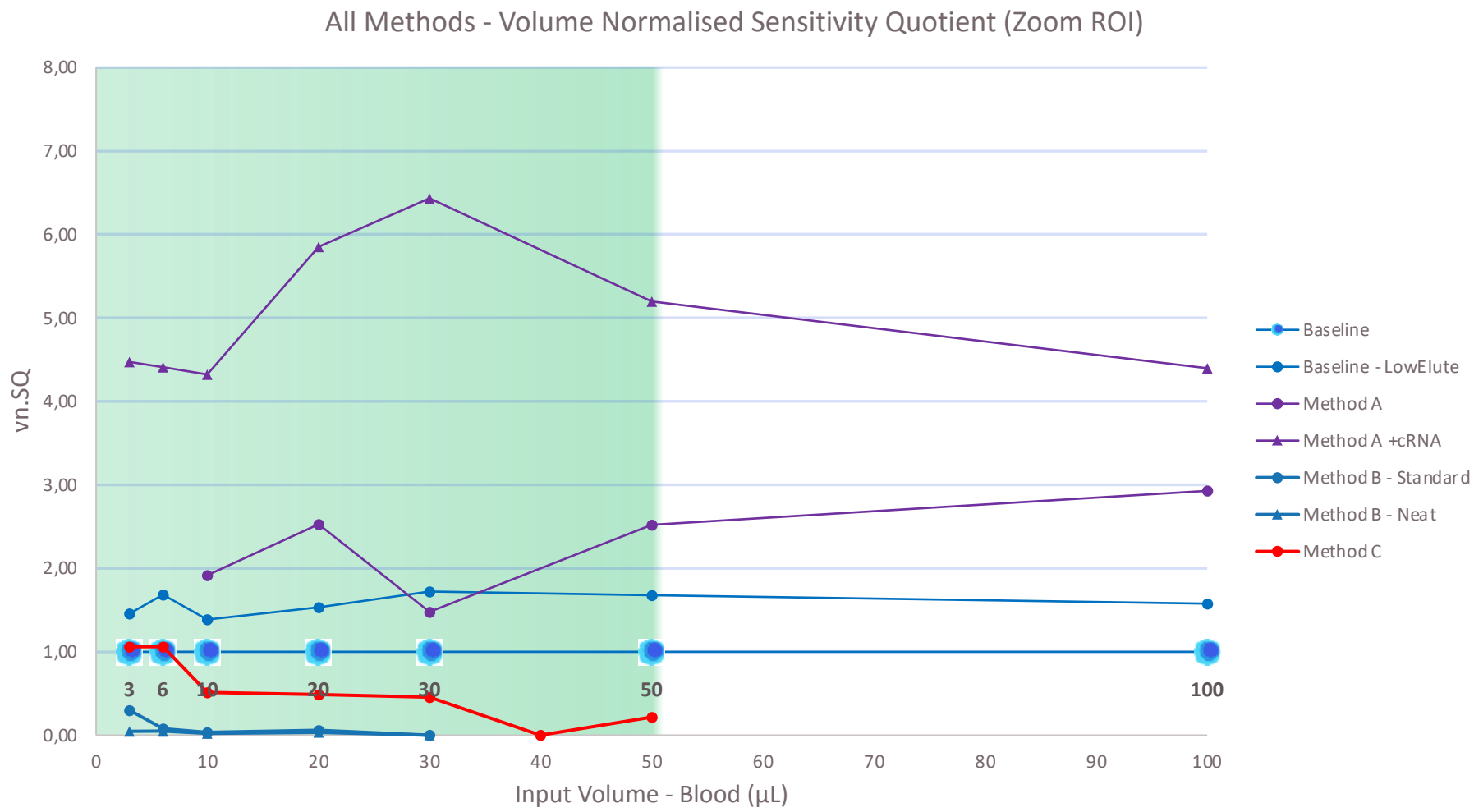
- The Baseline method shows lower %Recovery with a lower elution volume.
- Methods B and C have generally low Recovery% even where some parameters for Method B were adjusted towards use of the sample neat.
- Method A shows somewhat comparable %Recovery to the baseline method and shows high recovery upon the addition of carrier RNA to the extraction.

Results – All methods



- The Baseline method shows better sensitivity with a lower elution volume.
- Method B demonstrates low sensitivity at all volumes tested.
- Method C shows poor sensitivity and only matches the baseline at lowest volumes.
- Method A shows superior sensitivity to the baseline method and, upon the addition of carrier RNA, shows comparable sensitivity, at 50 µL, to a 220µL sample on the baseline method.

Results – Normalized Sensitivity



- Reduction in elution volume for the Baseline method provides a roughly 50% boost in sensitivity
- Sensitivity is increased across the range for Method A by around 2-fold, and more on addition of carrier RNA.
- Method B has low sensitivity across the range.
- Method C has low sensitivity across the range.

Conclusions and Future Considerations

Future Considerations and Conclusions

Conclusions

- Our standard method is consistent but not suitable
- **Front runner:** Method A
- The Preliminary assessments provide a good starting point
  - We have confidence that we will be able to offer our clients excellent support in future microsampling analyses.

Where do we want to go next?

- Advance our investigation of promising methods
- Assess additional kits and methods of extraction
- Assess changes to qPCR parameters to improve sensitivity
- Additional spiking assessment with viral vector
- Look at the samples from real microsamplers working with our Labcorp CLS
- Look at dried blood spot samples
- Review additional donors and other vector shedding matrices

Thank You!

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