Epidemiological Data Differences between Gel-purified vs. DNA-purified Oral Polio Vaccine in Environmental Samples

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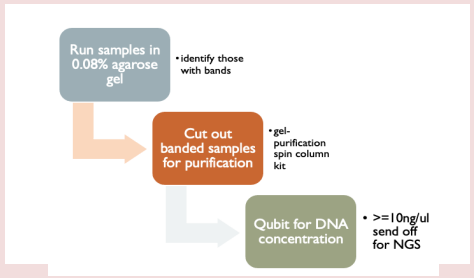
BACKGROUND

As wild poliovirus is eradicated, preventing circulation of vaccine-derived poliovirus is top priority. Our lab developed real-time multiplex PCR assays and deep sequencing methodology to detect and characterize OPV strains from stool samples. The method requires gel purification of PCR product created from viral RNA in stool samples. However, the process filters out a significant portion of samples. Here, we compare gel- vs. DNA- purification and sample retainment for downstream analysis.

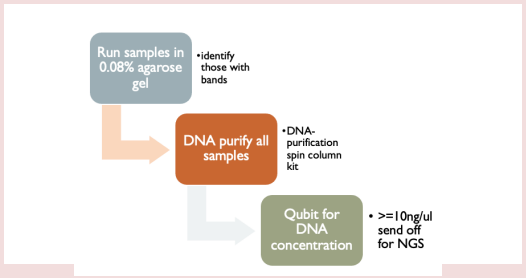
METHODS

554 stool samples qPCR positive for at least one OPV serotype were used in this comparison. There are 268 serotype 1 (S1) isolates, 405 serotype 2 (S2) isolates, and 318 serotype 3 (S3) isolates. PCR amplicons created from viral RNA ran through a 0.08% agarose gel to identity presence of the ~3.5kb amplicon of interest. PCR amplicons then underwent either a gel-purification spin column kit or a DNA purification spin column kit. Samples with DNA concentration >=10ng/uL in the elution product is required for NGS.

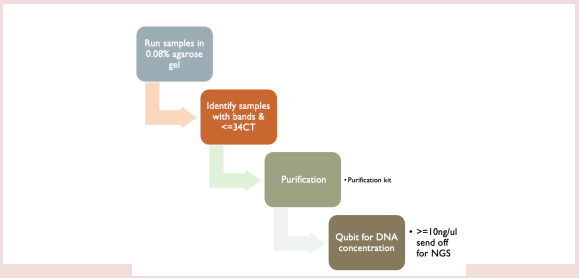
METHODS



Gel Purification Workflow



DNA Purification Workflow



Suggested Purification Workflow

Graph 1: Different PCR amplicon purification workflows

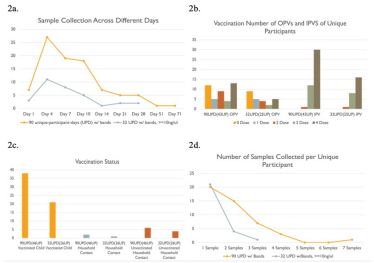
RESULTS

	UPD w/band	UPD w/band, >=10ng/ul	Coverage	UPD w/band <=34CT	UPD w/band <=34CT, >=10ng/ul
S2 DNA	90	90 (100%)		73	73 (100%)
S2 Gel	90	32 (35.56%)		75	31 (41.33%)
Sample loss from Gel-purification		64.44%			58.67%

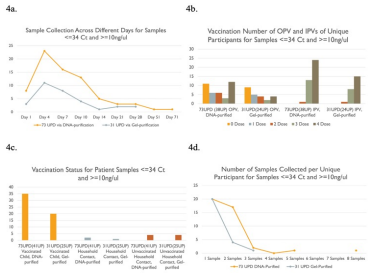
Table 1: Purification outcome of 168 S2 unique-participant-day (UPD) samples via gel-purification and DNA-purification workflows with respective NGS coverage aligned preliminarily with S3 genome

	S3 DNA Purification	S2 Gel Purification	Samples Filtered out by gel-purification
W/band	99	90	
W/band + >=10ng/ul	99 (100%)	32 (35.56%)	64.44%
W/band + >=10ng/ul + >100C	65 (65.66%)	28 (31.11%)	34.55%
Avg Genomic Coverage (aligned with S3 genome)	204.79 (99)	194.45 (32)	

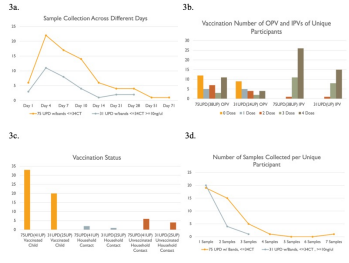
Table 2: Banded samples count of DNA-purified S3 samples (99) vs. gel-purified S2 samples (90) with DNA concentration >=10ng/ul and mean genomic coverage (C) aligned preliminarily to S3 genome.



Graph 2: Demographic information of patient sample loss on 168 S2 samples via gel-purification workflow. 1b. OPV and IPV vaccination information only avaliable for 43/46 unique participants (UP) for 90 banded samples and 25/26 UPs for 32 gel-purified banded samples with DNA concentration >=10ng/ul.



Graph 4: Demographic information of patient sample loss on 168 S2 samples from suggested workflow using gel-purification vs DNA-purification. 3b. OPV and IPV vaccination information only avaliable for 38/41 unique participants (UP) for 73 DNA-purified banded samples with <= 34CT and DNA concentration >=10ng/ul and 25/26 UPs for 31 gel-purified banded samples with <=34CT and DNA concentration >=10ng/ul.



Graph 3: Demographic information of patient sample loss on 168 S2 samples via suggested purification workflow. 2b. OPV and IPV vaccination information only avaliable for 38/41 unique participants (UP) for 75 gel-purified banded samples with have <=34 CT value, and 24/25 UPs for 31 gel-purified banded samples with <=34 CT value and DNA concentration >10ng/ul.

Comparing 168 S2 samples gel-purified vs. DNA-purified, of 90 bands identified with both protocols, 100% of DNA-purified samples and 35.6% of the gel-purified samples had DNA concentration >=10ng/ul (required for NGS). 41.3% of banded samples with <=34CT had >=10ng/ul for gel-purified samples vs. 100% for DNA-purified samples.

Among samples processed via gel purification, the median number of samples per positive participant dropped from 2 (IQR=1-3) to 1 (IQR=1-1) and the number of OPV vaccinated children 38 (82.6%), their household contacts 2 (4.3%), and community contacts 6 (13%) to 21 (80.7%) OPV vaccinated children, 1 (3.8%) household contacts, and 4 (15.3%) community contacts. A shift in OPV and IPV dosing proportion was also observed with gel-purification.

Mapped preliminarily against S3 reference genome, the 32 banded S2 gel-purified amplicons sent for NGS had mean coverage of 194.45, and the 99 banded S3 DNA-purified amplicons sent for NGS had mean coverage of 204.79.

There was a negative correlation between increasing CT and coverage and a weak positive correlation between DNA concentration and coverage. Gel purification of 10 bands took on average 4-5 hours while DNA-purification was about 30 minutes.

CONCLUSION

Gel-purification causes a reduction in sample numbers and variation, causing an incomplete data set and data misrepresentation for downstream analysis, particularly a reduction of samples for within host variation analysis. It is also significantly more time consuming than DNA-purification.

Aligned to the S3 genome, there is significant sample loss while genomic coverage is similar. Further analysis mapping samples against respective serotypes are needed for clearer understanding.

Given that DNA concentration of >=10ng/ul are needed for NGS and a negative correlation between increasing CT and genomic coverage, DNA purification of banded samples with <=34CT may be most efficient for NGS: retaining the most samples while ensuring quality of samples for genmoic analysis in a shorter amount of time.

NEXT STEPS

Align each serotype with respective reference genome and analyze the purification workflows with genomic coverage data.

Finish processing all samples for analysis of a bigger data set.

ACKNOWLEDGEMENTS

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