Comparing DNA Extraction Methods for the LymphoTrack® IVD *TRG* Assay

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Introduction

The LymphoTrack IVD *TRG* Assay is an investigational sequencing based assay being validated to identify clonal *TRG* V-J rearrangements, the associated V-J region DNA sequences and to provide the frequency distribution of the V-J segment utilization. The assay tests DNA extracted from peripheral blood (PB) to determine the presence or absence of T-cell clonality in subjects. DNA is amplified via PCR using individual master mixes with primers that target family-specific V and conserved J regions of the *TRG* gene and incorporate adapter sequences with up to 24 individual barcodes. Isolation of high-quality, inhibitor-free, double stranded DNA from peripheral blood samples is essential for successful PCR followed by sequencing. In order to provide users with variety of DNA isolation methods, 3 commercially available DNA extraction kits were evaluated for use with our LymphoTrack IVD *TRG* Assay.

Materials and Methods

- The LymphoTrack IVD *TRG* Assay is being evaluated for identification of clonality in *TRG* gene rearrangements in individuals with suspect T-cell clonality. The assay targets the human T-cell receptor gamma (*TRG*) gene locus. The PCR primers are designed to target (V) genes (Family I, II, III, and IV), and the joining (J) gene segments where gene rearrangement occurs during T-cell development (**Figure 1**).

Figure 1. Schematic diagram of the human T-cell receptor gamma gene

- The sample type for the LymphoTrack IVD *TRG* Assay is DNA extracted from peripheral whole blood. DNA is amplified via PCR; PCR products are quantified, pooled, and loaded into a MiSeqDx cartridge which contains all of the reagents required for cluster generation and Sequencing by Synthesis. The sequence data is compiled by the MiSeqDx Instrument into FASTQ files. Invivoscribe's LymphoTrack IVD Software is intended to perform automated data extraction, analysis, and generate reports from the input FASTQ files for clonality determination using the LymphoTrack IVD *TRG* Assay – MiSeqDx. **Figure 2** below represents workflow summary.

Figure 2. LymphoTrack IVD *TRG* Assay – MiSeqDx Workflow summary

- Three commercially available DNA extraction kits were evaluated in this study: (1) representing magnetic beads using QIAGEN MagAttract HMW DNA kit, (2) precipitation using JetFlex Genomic DNA Purification kit and (3) silica column using QIAGEN, QIACube method. Each kit was tested twice to extract DNA from 3 *TRG* clonal low positive contrived samples (IVS-0053; IVS-0061; IVS-0075) made by blending clonal cells of known cell input with pooled negative peripheral blood and 3 *TRG* clonal negative peripheral blood samples (PB1; PB2; PB3) by 2 operators. Each DNA sample was tested by the LymphoTrack IVD *TRG* Assay in duplicate (*TRG* Run) and sequenced together on a single MiSeqDx instrument. The study design is illustrated in **Figure 3.**

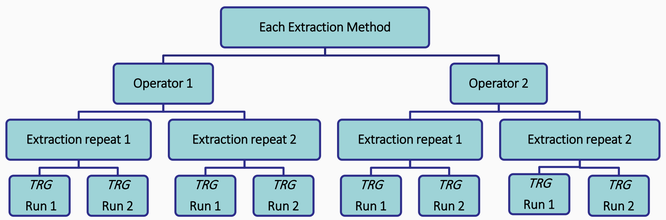

Figure 3. Flowchart of Extraction Methods Study

- Total DNA yield, quantified LymphoTrack IVD *TRG* Assay PCR product and final clonal results (%Reads) were compared across the 3 extraction methods.

Discussion and Conclusions

- All 3 evaluated DNA extraction kits provided enough DNA (≥10 ng/µL) for the LymphoTrack IVD *TRG* Assay. The precipitation method (PPT) produced the highest total DNA yield while the column method produced the least amount of DNA. Data is presented in **Table 1**.
- The generated PCR product yields were comparable among these 3 extraction methods. Average amplicon concentrations are presented in **Table 1**. No PCR inhibition was observed for any of the extraction methods.
- The *TRG* clonality calls generated using the LymphoTrack IVD *TRG* Assay - MiSeqDx were 100% concordant for all contrived samples processed using all DNA extraction kits **(Table 2)**.
- Variance components analysis was used to estimate the variability of %Reads due to the extraction methods, operator and random error for low positive contrived samples. The difference among extraction methods contributed no more than 5% of total variability.
- The variability in reported %Reads for positive samples (i.e. frequency of detected *TRG* rearrangement) was small with overall CV% ranging from 6.5% to 13.6% across all V-J rearrangements.
- Comparable results were generated using 3 DNA extraction kits with the LymphoTrack IVD *TRG* Assay.
- This study demonstrated that LymphoTrack IVD *TRG* Assay performance is independent of DNA extraction method.

Results

- DNA yield and amplicon concentration.

DNA yield was quantified using Qubit dSDNA BR Assay kit. Amplicon concentration determined by the Agilent Bioanalyzer using DNA1000 kit. Data summarized in **Table 1** below.

Table 1. Efficiency with respect of DNA Yield. Average Amplicon Concentration per Extraction Methods

Extraction Method	Extraction input (µL)	Cells input per one Extraction	DNA Yield after Extraction (DNA ng)	Extraction Efficiency (%)	Avg. amplicon concentration (nm) N=168
Silica column	200	1.00E+06	2346	36.1	23.2
Precipitation	300	1.52E+06	6209	63.0	20.6
Mag Beads	200	1.00E+06	3292	50.6	20.0

- Sequencing results (% Reads).

Each extraction method consistently detected expected *TRG* V-J rearrangements associated with V-J region DNA sequences. The distribution of % Reads per each clonal low positive contrived samples V-J rearrangements and negative PB samples was similar between the 3 extraction methods as shown in **Figure 4.**

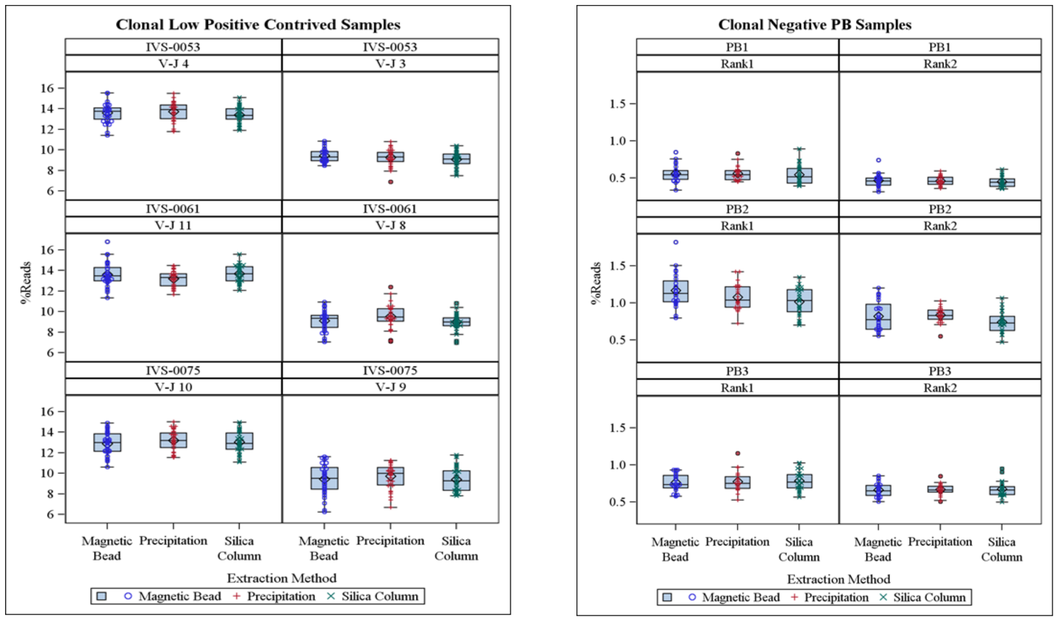

Figure 4. Distribution of %Reads for the 3 DNA extraction methods (low positive contrived samples and healthy PB samples)

- All samples showed 100% concordance with expected clonality results data summarized in **Table 2** below.

Table 2. DNA Extraction Method Results

Extraction Method	Sample Type	Concordance	Within-Method Concordance	Overall Concordance
Silica column	Negative	100% (72/72)	100% (168/168)	100% (504/504)
	Low Positive	100% (96/96)		
Precipitation	Negative	100% (72/72)	100% (168/168)	
	Low Positive	100% (96/96)		
Magnetic bead	Negative	100% (72/72)	100% (168/168)	
	Low Positive	100% (96/96)		

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* LymphoTrack IVD *TRG* Assay for Investigational Use Only. The performance characteristics of this product have not been established.

AMP, November 05-09 2019, Baltimore, Maryland , USA