Standardized Sample Preparation For Multiple Sample Matrices

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ABSTRACT

There are many different methods to process different sample matrices. This can lead to confusion and frustration for researchers working with multiple sample types. This abstract describes the MagMAX™ Pathogen RNA/DNA kit which is designed to achieve a more standardized solution so labs can order just one kit for a variety of sample matrices as well as different input volumes for each sample. This will allow labs to work with many different sample matrices using the same chemistry and instrumentation. Sample matrices that can be processed using this kit include blood, serum, swabs, semen, feces, cell supernatant
and others, from a sample volume of 50 µL up to 400 µL.

INTRODUCTION

Sample preparation is an integral component of the pathogenic nucleic acid amplification workflow. Coordinated development of workflows with consideration of the sample matrix, nucleic acid of interest, as well as format of downstream analysis can have a profound impact on the successful amplification of target nucleic acids from animal sourced matrices. Effective sample preparation from a wide variety of sample matrices is critical to accurate testing, and the development of sample preparation methodologies in the context of the entire testing workflow is key to ensure optimal performance.

MATERIALS AND METHODS

Porcine serum samples of varying concentrations were acquired and used for this experiment. They were true samples from the field and not purified serum.
For the purpose of this experiment, PRRSV (Porcine Reproductive and Respiratory Syndrome Virus) infected serum was used at 4 different viral shedding statuses. Samples that were determined to be high shedders had a C_T value between 15 and 20. Medium shedders had a CT value between 20 and 25, low shedders has a CT value close to 30 and negatives showed no viral infection.
Each shedding category contained three samples and each sample was processed in replicates of four. This resulted in 96 samples. These 96 samples were processed using three different sample preparation kits. For the purpose of this experiment, the MagMAX™ Pathogen RNA/DNA kit was compared against a sample preparation kit from Competitor A and one from Competitor B. The following table represents the experimental setup for the sample preparation part of the experiment.

			1	2	3	4	5	6	7	8	9	10	11	12
Life	um	A	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		B	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		C	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		D	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3

			1	2	3	4	5	6	7	8	9	10	11	12
Competitor A		A	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		B	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		C	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		D	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3

			1	2	3	4	5	6	7	8	9	10	11	12
Competitor B		A	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		B	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		C	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3
		D	H1	H2	H3	M1	M2	M3	L1	L2	L3	N1	N2	N3

Table 1: Sample Preparation setup: H = High Shedder, M = Medium Shedder, L = Low Shedder and N = Negative. Numbers 1-3 indicate sample number.

Life Technologies	Competitor A	Competitor B
200 µL Sample	100 µL Sample	200 µL Sample
500 µL Lysis Solution	600 µL Lysis Solution	200 µL Lysis Buffer
300 µL Wash 1 X 2	700 µL Wash 1	600 µL Binding Buffer
450 µL Wash 2 X 2	500 µL Wash 2	500 µL Wash 1
90 µL Elution	500 µL Wash 3	500 µL Wash 2
	90 µL Elution	550 µL Wash 3
		90 µL Elution

Table 2: Protocol Specifications: The three protocols were fairly similar in terms of sample input and all used the same elution volume. Therefore, no normalization of data was required. All protocols were run using the MagMAX Express-96 instrument programmed with the individual kit protocols.

RESULTS

Figure 1. Sample Prep Kit comparison for Porcine Serum

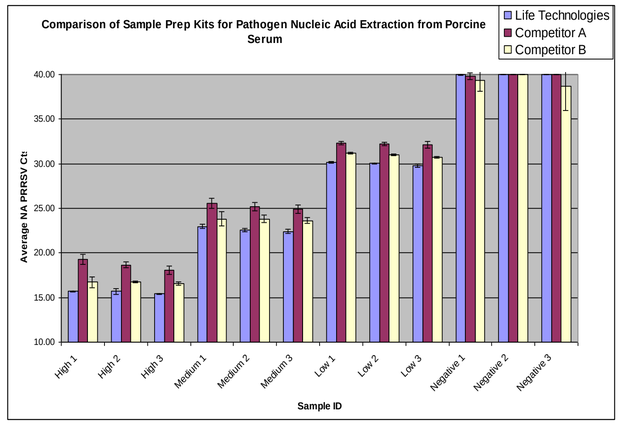


Figure 1: Comparison of Sample Preparation Kits: From the results above, it is clear that the MagMAX™ Pathogen RNA/DNA kit performs better than the two competitors.

Figure 2. MagMAX™ Pathogen RNA/DNA kit comparison with spin columns

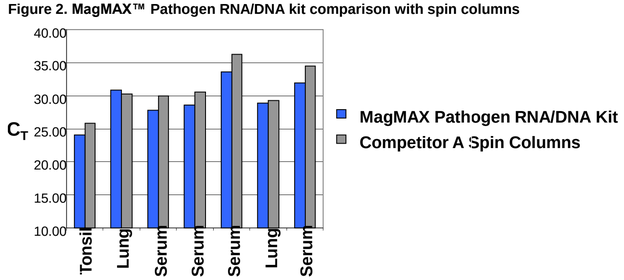


Figure 2: Purification of viral RNA from serum and tissue homogenates. RNA was purified from PRRSV-positive tissue homogenates and serum using the MagMAX™ Pathogen RNA/DNA Kit (50ul sample input) and a viral RNA isolation kit from Competitor A (140ul sample input). RNA was analyzed by qRT-PCR. Even with ~3 fold lower sample input, pathogen nucleic acid amplification was as sensitive (equivalent C_T) or more sensitive (lower C_T) using nucleic acid purified with the MagMAX™ Pathogen RNA/DNA Kit. This is consistent with better washing and PCR inhibitor removal by MagMAX™.

Figure 3. Multiple sample matrices can be processed using the MagMAX™ Pathogen RNA/DNA Kit

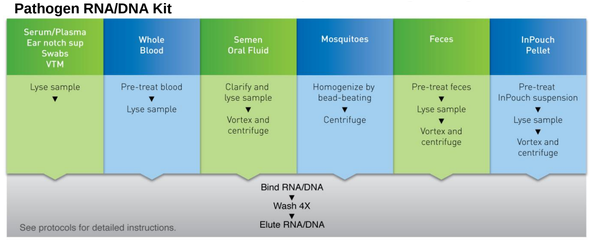


Figure 3: The kit is designed for volumes ranging from 50 µL all the way up to 400 µL.. Depending on the sample type, there may be additional pre-processing required. However, this step does not significantly add to the processing time.

Figure 4. MagMAX™ Workflow

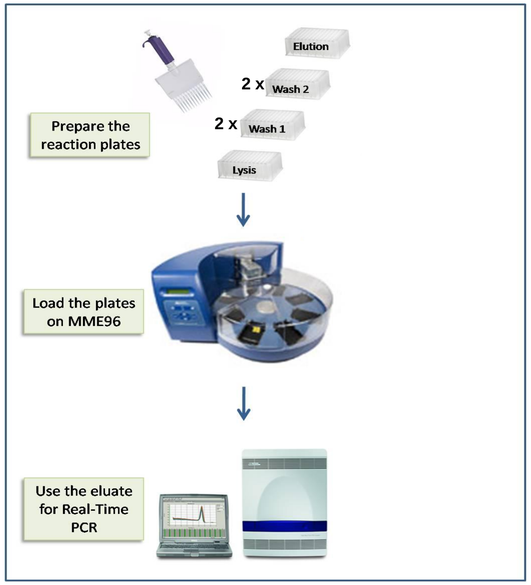
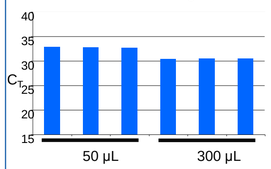
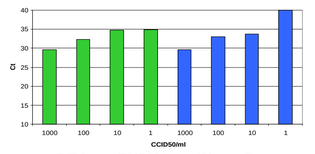


Figure 4: Workflow for processing various sample types using the MagMAX™ Pathogen RNA/DNA Kit. It takes approximately 45 minutes to purify nucleic acid from 96 samples including adding individually to the 96 well plate. It takes another 1.5 hours for the PCR reaction. Sample to signal is therefore cut down to approximately 3 hours.

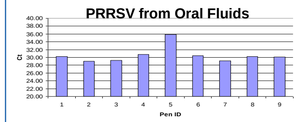
Figure 5: Isolations from different sample matrices

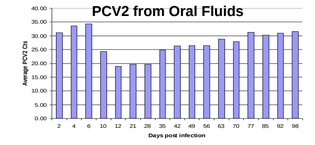


BVDV from Pooled Serum



BTV and EHDV from Whole Blood





CONCLUSIONS

CONVENIENCE FLEXIBILITY EFFICIENCY	HAVE CONFIDENCE IN YOUR RESULTS
• One kit for – Wide range of sample types – RNA & DNA purification – Viruses, bacteria, parasites – Low and high input volumes • Perform automated or manual processing • Run multiple sample types at the same time • Fast workflow • Easy to learn and use	• Reduce false-negative results due to effective PCR inhibitor removal – Magnetic particles have better nucleic acid binding and washing efficiencies • Minimize cross contamination using optimized protocols • Easy automation to minimize sample handling

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