Sample prep

Versatile solutions for infectious disease research with the MagMAX Prime Viral/Pathogen NA Isolation Kit

The Applied Biosystems™ MagMAX™ Prime Viral/Pathogen NA Isolation Kit, prefilled plates, and accessories can be used to:

- Process multiple sample types across various applications, including:
  - Respiratory tract infections (RTIs)
  - Sexually transmitted infections (STIs)
  - Urinary tract infections (UTIs)
  - Bloodborne infections (BBIs)
  - Gastrointestinal infections (GIs)
- Extract nucleic acid from various pathogens, including viruses, bacteria (gram-negative and gram-positive), yeasts, fungi, and parasites
- Enable simple, automated workflows using Thermo Scientific™ KingFisher™ instruments:
  - Basic workflow
  - Advanced lysis workflow
  - Advanced stool workflow

Introduction

Traditionally, bacterial culture and immunoassays have been employed to screen for infectious microbes within clinical research. These methods were disrupted by the introduction of molecular technologies like qPCR, which is faster, more sensitive, and highly specific. To match the expeditious turnaround provided with molecular screening for clinical research, microbial profiling, and surveillance, there is a need for streamlined, automated, and versatile sample preparation solutions suitable for various infectious disease applications, including RTI, STI, UTI, BBI, and GI clinical research.

The MagMAX Prime Viral/Pathogen NA Isolation Kit is designed for infectious disease research across various applications, sample types, and pathogens [1,2].

The MagMAX Prime product line includes:

- The MagMAX Prime Viral/Pathogen NA Isolation Kit, for users who desire the flexibility of user-prepared formats
- The MagMAX Prime Viral/Pathogen NA Isolation Kit, prefilled plate format, for users who desire the convenience of prefilled reagent plates to save time and effort
- MagMAX Prime accessories to enable users to:
  - Process gram-positive bacteria and fungi with the Applied Biosystems™ MagMAX™ Prime Viral/Pathogen G+ Bacterial and Fungal Lysis Buffer
  - Process stool with the Applied Biosystems™ MagMAX™ Prime Viral/Pathogen Stool Lysis Buffer and Bead Beating Tubes

Table 1 lists the contents of the MagMAX Prime Viral/Pathogen kit along with the accessory options. For more information on the basic and advanced extraction workflows of the MagMAX Prime Viral/Pathogen kit or prefilled plate options, see thermofisher.com/mvpprime.

Table 1. Contents of the MagMAX Prime Viral/Pathogen NA Isolation Kit.

Component	Storage
Basic components	
Binding Solution	15°C to 25°C
Wash Solution	
Binding Beads	
Proteinase K	
Elution Solution	
Proteinase K Dye	
Advanced accessory components	
MagMAX Prime Viral/Pathogen G+ Bacterial and Fungal Lysis Buffer	–25°C to –15°C
MagMAX Prime Viral/Pathogen Stool Lysis Buffer	15°C to 25°C
MagMAX Prime Viral/Pathogen Bead Beating Tubes	

Background

The MagMAX Prime Viral/Pathogen product line employs MagMAX bead-based technology for simple and effective automation on KingFisher instruments, combining the best qualities from existing MagMAX products like the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (RUO), MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit, and MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit. Table 2 highlights differences in the MagMAX kits across pathogen, application, and sample compatibilities.

In this technical note, we review various workflow solutions with the MagMAX Prime Viral/Pathogen kit that meet clinical research needs across five infectious disease applications: RTIs, STIs, UTIs, BBIs, and GIs. Relevant sample types like dried blood spot (DBS), plasma, anterior nasal swab, vaginal swab, urine, and raw stool were evaluated, utilizing metrics including extraction effectiveness, workflow timing and reproducibility, and risk of cross-contamination.

Table 2. Comparison of pathogen, application, and sample compatibilities of MagMAX Viral/Pathogen kits.

	MagMAX Viral/ Pathogen kit	MagMAX Viral/ Pathogen II kit	MagMAX Viral/ Pathogen Ultra kit	MagMAX Microbiome Ultra kit	MagMAX Prime Viral/Pathogen kit
Pathogens					
DNA viruses	●	●	●	●	●
RNA viruses	●	●	●	●	●
Gram-negative bacteria	●	●	●	●	●
Gram-positive bacteria	○	○	●	●	●
Yeasts and fungi	○	○	●	●	●
Parasites	○	○	●	●	●
Common applications					
RTIs	●	●	●	○	●
STIs, UTIs	●	●	●	○	●
Wound microorganisms	○	○	●	○	●
BBIs	●	○	●	○	●
GIs	○	○	○	●	●
Sample types					
Biofluids (plasma, serum, urine, bronchoalveolar lavage (BAL), saliva)	●	●	●	●	●
Swabs in transport media	●	●	●	●	●
DBSs	●	○	○	○	●
Stool and fecal swabs	○	○	○	●	●

Key	
Contains feature for extraction	●
Does not contain feature for extraction	○

Experimental approach

Overview

The MagMAX Prime Viral/Pathogen kit was assessed for its ability to extract nucleic acids from various organisms and different representative sample types. Organisms were chosen and samples were contrived based on their ability to challenge the extraction workflows, along with their relevance to infectious disease applications.

Three different workflows (basic, advanced lysis, and advanced stool) were utilized in these studies. Table 3 summarizes the studies performed.

Table 3. High-level summary of study design for evaluation of the MagMAX Prime Viral/Pathogen kit.

Study number	Evaluation	Workflow	Kit format	Total number of extractions
1	Versatility and effectiveness	Basic, advanced lysis, advanced stool	User-prepared plates	19
2	Workflow timing and reproducibility	Basic	User-prepared and prefilled plates	108
		Basic, advanced lysis, advanced stool		10
3	Cross-contamination risk	Basic		12

Versatility and effectiveness

Basic workflow

The basic workflow was utilized to extract nucleic acids of representative pathogens from relevant sample types in BBI, RTI, STI, and UTI applications. Figure 1 details the representative basic workflow that does not need MagMAX Prime Viral/Pathogen kit accessories for enzymatic or mechanical lysis sample processing. Across the basic workflow extractions, a total of 57 replicates encompassing various contrived samples were evaluated for percent positive amplification as an indicator of extraction effectiveness.

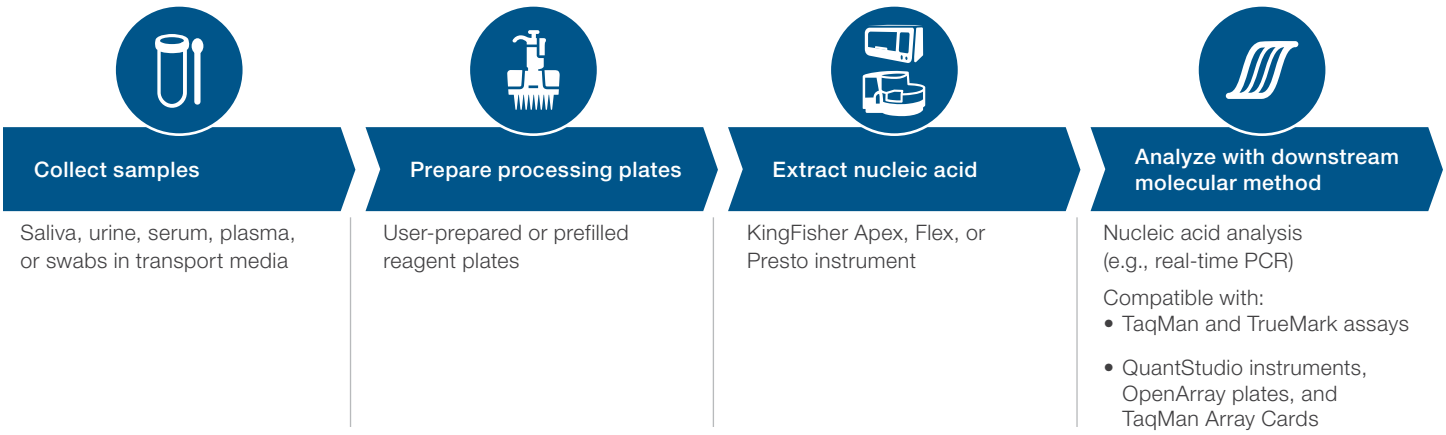

Figure 1. Basic workflow of the MagMAX Prime Viral/Pathogen kit for viruses and gram-negative and easy-to-lyse bacteria.

Table 4 details information on the contrived samples and accessories needed for the basic workflow evaluated. All samples leveraging the basic workflow were processed on the Thermo Scientific™ KingFisher™ Flex Purification System using the Prime_FLX.bdz script.

Table 4. Contrived samples and accessories used for the basic workflow of the MagMAX Prime Viral/Pathogen kit.

Common application	Pathogen type	Organism	Sample type	Quantity contrived per extraction	Accessories
BBI	Virus	HIV-1	DBS	12,000 copies	MagMAX Viral/Pathogen DBS Lysis Solution (Cat. No. A53651)
			Plasma	12,000 copies	None
RTI	Virus	Influenza A virus	Anterior nasal swab	1.6 $TCID_{50}$	None
	Gram-positive bacterium	*Staphylococcus aureus*	Anterior nasal swab	1,000 CFU	
STI	Virus	HSV-1	Vaginal swab in Amies medium	10,000 copies	None
	Gram-negative bacterium	*Mycoplasma hominis*	Vaginal swab in Amies medium	1,000 copies	
			Urine	1,000 copies	
STI, UTI	Parasite	*Trichomonas vaginalis*	Vaginal swab in Amies medium	680 copies	None
			Urine	680 copies	

Advanced lysis workflow

The advanced lysis workflow is used for difficult-to-lyse gram-positive bacterial and fungal samples with the help of the accessory MagMAX Prime Viral/Pathogen G+ Bacterial and Fungal Lysis Buffer. Figure 2 describes the advanced lysis workflow. A total of 24 replicates across various organisms were evaluated for percent positive amplification. The samples were contrived with organisms such as *Candida albicans* and *Streptococcus agalactiae* in sample types such as vaginal swabs in Amies medium and urine. Table 5 shows details of the samples' composition. All advanced lysis workflow samples were processed on the KingFisher Flex instrument using the Prime_GPB_Fungi_FLX.bdz script.

* Enzymatic treatment with MagMAX Prime Viral/Pathgen G+ Bacterial and Fungal Lysis Buffer is automated on the KingFisher Flex instrument with the Prime_GPB_Fungi_FLX.bdz script and on the KingFisher Apex instrument with the Prime_GPB_Fungi_APX.kfx script, with a pause during the script after the enzymatic treatment to add the lysis/binding and bead mix (refer to Pub. No. MAN0029683).

Figure 2. Advanced lysis workflow of the MagMAX Prime Viral/Pathogen kit for difficult-to-lyse gram-positive bacterial and fungal targets.

Table 5. Contrived samples and accessories used for the advanced lysis workflow of the MagMAX Prime Viral/ Pathogen kit.

Common application	Pathogen type	Organism	Sample type	Amount contrived	Accessories
STI, UTI	Fungus	*Candida albicans*	Vaginal swab in Amies medium	10,000 copies	MagMAX Prime Viral/Pathogen G+ Bacterial and Fungal Lysis Buffer
			Urine		
	Gram-positive bacterium	*Streptococcus agalactiae*	Vaginal swab in Amies medium		
			Urine		

Advanced stool workflow

The advanced stool workflow was designed specifically for GI applications utilizing stool sample types. A mechanical lysis workflow prior to automated extraction is recommended for all stool samples, regardless of pathogen, to ensure high-quality nucleic acid isolation. This workflow requires the MagMAX Prime Viral/Pathogen Stool Lysis Buffer and MagMAX Prime Viral/Pathogen Bead Beating Tubes as accessories. Figure 3 describes the advanced stool workflow. Across the advanced stool workflow extractions, a total of 12 replicates contrived with various organisms were evaluated for percent positive amplification. Table 6 describes the contrived sample information and accessory needs for the workflow evaluated. *Cryptosporidium* and norovirus were nonenumerated and spiked volumetrically to obtain expected concentrations and amplification. All GI samples were processed on the KingFisher Flex instrument using the Prime_GI_FLX.bdz script.

* Use MagMAX Prime Viral/Pathogen Stool Lysis Buffer and MagMAX Prime Viral/Pathogen Bead Beating Tubes for up-front mechanical lysis of fecal samples followed by extraction on the KingFisher Flex instrument with the Prime_GI_FLX.bdz script or the KingFisher Apex instrument with the Prime_GI_APX.kfx script.

Figure 3. Advanced stool workflow of the MagMAX Prime Viral/Pathogen Kit for up-front mechanical lysis of stool samples.

Table 6. Contrived samples and accessories used for the advanced stool workflow of the MagMAX Prime Viral/ Pathogen kit.

Common application	Pathogen type	Organism	Sample type	Amount contrived	Accessories
GI	Virus	Norovirus	Raw stool	Nonenumerated	MagMAX Prime Viral/Pathogen Stool Lysis Buffer, MagMAX Prime Viral/Pathogen Bead Beating Tubes
	Fungus	*Candida albicans*		10,000 copies	
	Gram-positive bacterium	*Listeria monocytogenes*		2,000 copies	
	Parasite	*Cryptosporidium*		Nonenumerated	

Workflow timing and reproducibility

To evaluate timing and reproducibility, a second study was performed utilizing the basic workflow with various contrived respiratory sample types. The study was performed across three independent MagMAX Prime Viral/Pathogen kit lots on the KingFisher Apex system. Six operators collectively conducted extractions from nasopharyngeal swabs stored in viral transport media, saliva, anterior nasal swabs, and sputum contrived with various viral and bacterial targets across six KingFisher Apex instruments. Completion time of the script on the KingFisher Apex instrument was recorded along with overall coefficients of variation (CVs) from lot to lot and run to run across 108 independent runs.

In addition to the 108 independent runs above, a follow-up study was performed on the KingFisher Flex instrument, comparing user-prepared and prefilled MagMAX Prime Viral/Pathogen formats utilizing different available workflows in the user guide (Pub. No. MAN0029683). The study spanned five different applications including the three workflow options and scripts. Across the different formats, time to completion of the workflows on the KingFisher instrument was recorded.

Cross-contamination

To evaluate the risk of cross-contamination, pooled nasopharyngeal swabs in viral transport medium (NPVTM) samples were contrived with inactivated SARS-CoV-2 at 10^5 PFU/mL and extracted with the basic workflow using user-filled and prefilled MagMAX Prime Viral/Pathogen kit formats. Extraction was performed on the KingFisher Apex instrument. A total of six runs across two operators and two KingFisher Apex instruments were performed for both 200 µL and 400 µL sample input volumes with the Prime_APX.kfx script.

A checkerboard pattern was utilized in each extraction, with NPVTM pools contrived with high-titer SARS-CoV-2 surrounded by NPVTM pools not contrived standing as a negative extraction control (Figure 4). Results were analyzed with Applied Biosystems™ TaqMan™ Assays targeting SARS-CoV-2, on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System using a standard 0.2 mL block for amplification. Each extraction event had a potential of 82 false amplifications resulting from amplification of the negative extraction control. Across all extractions, there were a total of 984 potential false amplifications by qPCR that could be related to potential contamination from well to well in extraction of nucleic acid. Percent false amplification was analyzed.

	1	2	3	4	5	6	7	8	9	10	11	12
A					■						■	
B		■						■				
C												
D					■						■	
E		■						■				
F												
G					■						■	
H		■						■				

Key	
■	Contrived high-titer SARS-CoV-2
	Negative extraction control

Figure 4. Layout for assessment of cross-contamination in high-titer sample extraction, with the basic workflow of the MagMAX Prime Viral/Pathogen kit on the KingFisher Apex instrument.

MagMAX Prime Viral/Pathogen kit reagents

Results and discussion

Versatility and effectiveness

Contrived samples across BBI, RTI, STI, and UTI applications were processed utilizing the basic workflow with a total of 57 replicates across various organisms, including viruses, bacteria, and parasites, with various sample types including DBS, plasma, anterior nasal swabs, urine, and vaginal swabs in Amies medium. The percent positive amplification rate was calculated by evaluating expected amplification from contrived specimens (Table 7). Percent positive amplification at 100% indicates extraction was successful from contrived samples and organisms with the basic workflow.

The advanced lysis workflow for STI and UTI applications was performed across fungal and gram-positive bacterial specimens, with a total of 24 sample replicates providing 100% positive amplification, indicating extraction was successful from contrived samples and organisms with this workflow.

The advanced stool workflow for GI applications was performed across fungal, parasitic, gram-positive bacterial, and viral stool specimens, with a 100% positive amplification rate indicating extraction was successful from contrived samples and organisms with this workflow.

The effectiveness of extraction across various organisms by workflow application of the MagMAX Prime Viral/Pathogen kit allows for versatility within each application (Table 7). Additional flexibility is provided by the ability to process sample inputs ranging from 200 µL to 400 µL on one plate within one extraction event. This study shows that various sample types, regardless of application, could be leveraged with just one extraction event, ultimately saving plastics, reagents, and time.

Table 7. Percent positive amplification by workflow and application of the MagMAX Prime Viral/Pathogen kit.

MagMAX Prime Viral/Pathogen kit workflow	MagMAX Prime Viral/Pathogen kit accessories	Application	Organism type	TaqMan Assay target	Sample type	Number of extraction replicates	Final amount per extraction	Amplification result
Basic	None	BBI	Virus	HIV-1	DBS*	3	12,000 copies	+
				HIV-1	Plasma	6	12,000 copies	
		RTI	Virus	Influenza A virus	Anterior nasal swab	6	1.6 $TCID_{50}$	
			Gram-positive bacterium	*Staphylococcus aureus*	Anterior nasal swab	6	1,000 CFU	
		STI, UTI	Virus	Herpes simplex virus	Urine	6	10,000 copies	
				Herpes simplex virus	Vaginal swab	6	10,000 copies	
			Gram-negative bacterium	*Mycoplasma hominis*	Vaginal swab	6	1,000 copies	
				Mycoplasma hominis	Urine	6	1,000 copies	
			Parasite	*Trichomonas vaginalis*	Vaginal swab	6	680 copies	
				Trichomonas vaginalis	Urine	6	680 copies	
Total number of extractions using the basic workflow for BBI, RTI, STI, and UTI applications						**57**	**Percent positive amplification**	**100%**
Advanced lysis	MagMAX Prime Viral/Pathogen G+ Bacterial and Fungal Lysis Buffer	STI, UTI	Fungus	*Candida albicans*	Urine	6	10,000 copies	+
				Candida albicans	Vaginal swab	6	10,000 copies	
			Gram-positive bacterium	*Streptococcus agalactiae*	Urine	6	10,000 copies	
				Streptococcus agalactiae	Vaginal swab	6	10,000 copies	
Total number of extractions using the advanced lysis workflow for STI and UTI applications						**24**	**Percent positive amplification**	**100%**
Advanced stool	MagMAX Prime Viral/Pathogen Stool Lysis Buffer and Bead Beating Tubes	GI	Fungus	*Candida albicans*	Stool	3	10,000 copies	+
			Parasite	*Cryptosporidium*	Stool	3	Nonenumerated	
			Gram-positive bacterium	*Listeria monocytogenes*	Stool	3	2,000 CFU	
			Virus	Norovirus	Stool	3	Nonenumerated	
Total number of extractions using the advanced stool workflow for GI applications						**12**	**Percent positive amplification**	**100%**

* DBS samples were lysed utilizing MagMAX Viral/Pathogen DBS Lysis Solution (Cat. No. A53651) as an up-front digestion accessory to the basic workflow.

Workflow timing and reproducibility

Across 108 independent extraction events, six operators, and six instruments, run-to-run, lot-to-lot, and well-to-well coefficients of variation (CVs) were analyzed for representative organisms, using respiratory targets processed with the basic workflow on the KingFisher Apex instrument. Run-to-run CVs across positive-sense single-stranded RNA (+ssRNA) and DNA viruses and representative bacteria were less than 3% (Table 8), suggesting minor variation from one extraction event to the next across various users and sample types. Lot-to-lot CVs for the same targets indicated less than 2%, and well-to-well CVs were evaluated at less than 4% across all microbial targets. In addition to CVs across microbial targets, the Prime_APX.kfx script run time was recorded across all 108 runs and ranged from 28 to 41 minutes across six independent KingFisher Apex instruments, with an average of 30 minutes.

Some run times varied by instrument and removal of eluate from the KingFisher Apex instrument post-run at the hold stage. The final hold stage was beneficial in these instances as it holds a cooling temperature to reduce risk of elution evaporation while maintaining eluate stability until the operator can come and remove the eluate for storage or for use in the downstream molecular application. In some cases, operators didn't receive their eluate for up to 11 minutes post-run without any impact to their downstream results.

Table 8. Percent CV with the basic workflow of the MagMAX Prime Viral/Pathogen kit utilizing respiratory sample types and microbial targets.

Organism type	Microbial target	Sample type	CV run-to-run	CV lot-to-lot	CV well-to-well
+ssRNA virus	SARS-CoV-2 Influenza A virus Influenza B virus Enterovirus	Anterior nasal swab NPVTM Saliva	2.58%	1.42%	2.30%
DNA virus	Adenovirus	Anterior nasal swab Saliva	0.10%	0.30%	3.80%
Gram-positive bacterium	*Staphylococcus aureus*	Anterior nasal swab NPVTM Saliva	1.63%	0.30%	1.10%
Gram-negative bacterium	*Bordetella pertussis*	NPVTM	2.50%	0.10%	2.20%
Acid-fast bacterium (AFB)	*Mycobacterium tuberculosis*	Sputum*	0.10%	0.20%	3.70%

* Sputum workflow requires preprocessing with sputasol before processing with the basic workflow of the MagMAX Prime Viral/Pathogen kit.

Script run times of the KingFisher Apex and KingFisher Flex instruments were recorded for user-prepared and prefilled reagent plates across the basic, advanced lysis, and advanced stool workflows. Figure 5 shows average times to completion of the script workflows across the different fill formats and KingFisher instruments. For the advanced stool workflow, prefilled reagent formatting is not available, so it was not evaluated. Timing for the basic, advanced lysis, and advanced stool workflow scripts averaged approximately 30 minutes, 51 minutes, and 45 minutes, respectively.

Minimal variation was observed between the KingFisher Flex and KingFisher Apex instruments, as well as between prefilled and user-prepared plate formats.

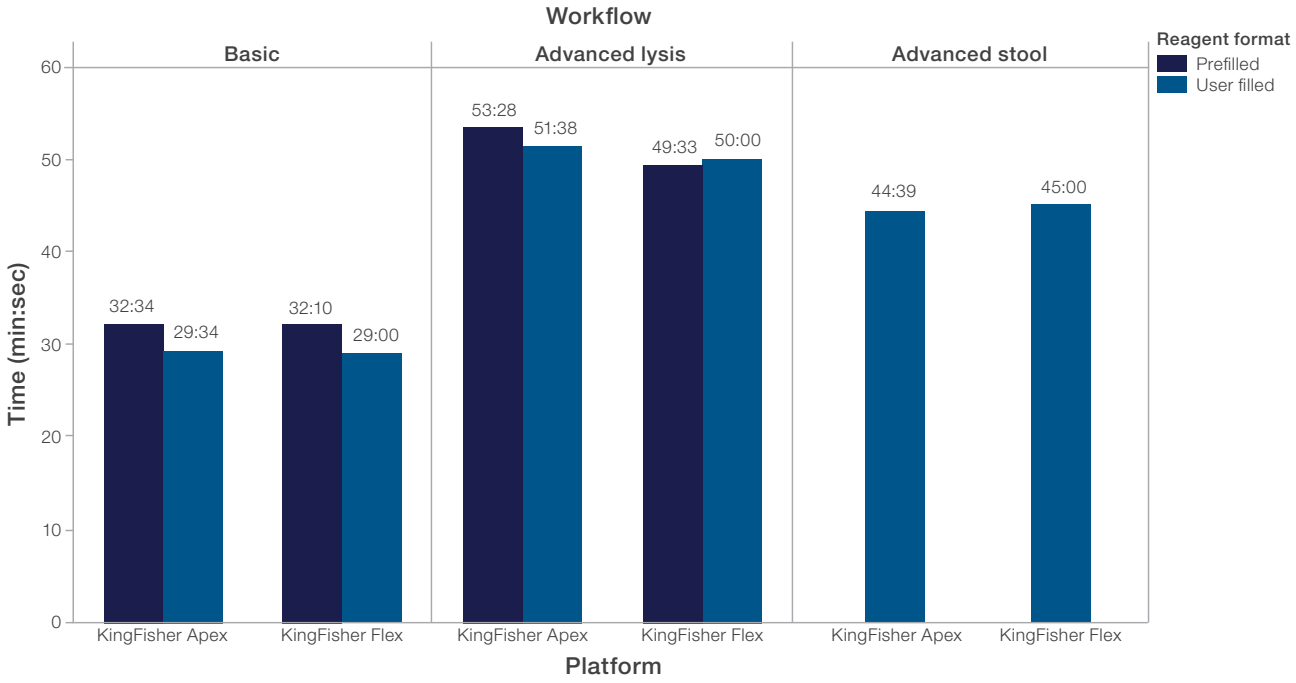

Figure 5. Time evaluation of basic and advanced workflows using the MagMAX Prime Viral/Pathogen kit with prefilled and user-filled reagent plates across KingFisher Flex and KingFisher Apex instruments.

Cross-contamination

Across all sample inputs and MagMAX Prime Viral/Pathogen reagent formats, cross-contamination risk was evaluated to be less than 0.5%, indicating minimal risk of well-to-well contamination whether the sample volume was 200 µL or 400 µL (Table 9).

Table 9. Summary of percent false amplification for the basic workflow of the MagMAX Prime Viral/Pathogen kit at varying sample inputs utilizing user-prepared and prefilled reagent plates.

Extraction number	MagMAX Prime Viral/ Pathogen kit workflow	Potential number of false amplifications	Percentage of false amplifications by workflow
User-prepared plates			
1	200 µL input	492	0.20%
2			
3			
4	400 µL input		
5			
6			
Prefilled plates			
1	200 µL input	492	0.00%
2			
3			
4	400 µL input		
5			
6			

Conclusions

Here we have demonstrated that the MagMAX Prime Viral/Pathogen kit is a versatile and reliable solution for extracting nucleic acids from various microbial targets and sample types with simple workflow options. The high performance and reproducibility of the MagMAX Prime Viral/Pathogen kit allow infectious disease researchers to use it as a standard approach for nucleic acid extraction with automation options for ease and convenience.

Authors

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References

1. Thermo Fisher Scientific (2023) Evaluation and characterization of microorganisms that cause sexually transmitted infections. Pub. No. COL35845.
2. Thermo Fisher Scientific (2023) Innovative MagMAX kits support multiple downstream qPCR workflows. Pub. No. COL121611.

Ordering information

Product	Cat. No.
MagMAX Prime Viral/Pathogen NA Isolation Kit	A58145
MagMAX Prime Viral/Pathogen NA Isolation Kit, prefilled plate format	A58146PF*
MagMAX Prime Viral/Pathogen G+ Bacterial and Fungal Lysis Buffer	A59053
MagMAX Prime Viral/Pathogen Stool Lysis Buffer	A58154
MagMAX Prime Viral/Pathogen Bead Beating Tubes	A58155
KingFisher Flex Purification System, 96 deep-well head and heat block	5400640
KingFisher Apex Purification System, 96 deep-well head and heat block	5400930

* Available in certain regions. Please reach out to your Thermo Fisher Scientific representative for more information.

Learn more at **thermofisher.com/mvpprime**