TANBEAD

Product Specification

Sample type	300 μL lysate serum or PBS suspension
Application	PCR, qPCR
Automatic process (min)	20 min
Features	Purify the high-quality nucleic acid from samples with diverse pathogens, including virus, bacteria, and fungi.

Purpose

TANBead® Nucleic Acid Extraction Kit 66P series (Super 665) is a new product mainly designed for the automatic isolation of DNA or RNA from a wide range of samples. The advantages of this 66P series kit compared with the original 665 series kit is that it does not require sample pretreatment and can extract viral, bacterial, and fungal nucleic acids from a variety of samples. Sample types include pure cultures of any pathogenic microorganisms (*e.g.*, bacteria, virus, fungi, and mycoplasma), clinical samples, and samples containing multiple pathogens with complex backgrounds (*e.g.*, urine, serum, blood, and swab). In this technical report, our purpose is to examine the performance of TANBead® 66P kit in extracting nucleic acids from pure and complex samples, and clinical samples of sexually transmitted infections (STIs) through quantitative real-time PCR (qPCR) analysis.

Materials and Methods

Pure samples

1. HPV16: 1,500 copies of AcroMetrix™ HPV-16 Genotype Control (Thermo Scientific) were spiked into the Universal Transport Medium® (UTM, COPAN Diagnostics).
2. SARS-CoV-2: 500 copies of AccuPlex™ SARS-CoV-2 Reference Materials (SeraCare) were spiked into the UTM.
3. HIV: 18.5 IU of artificial HIV lentiviruses (MDBio) were spiked into the UTM.
4. *Staphylococcus* sp., *Listeria* sp., *Salmonella* sp., *Pseudomonas aeruginosa*, and *Candida albicans*: 1 μL (OD600 = 1.0) of bacteria or fungi cultured in Luria-Bertani liquid medium were spiked into the UTM.

Complex samples

1. All mixed in serum, blood: 1 μL (OD600 = 1.0) of *C. albicans*, *Listeria* sp., *Salmonella* sp. 500 copies of *Neisseria gonorrhoeae* positive control (ZeptoMetrix) and $5x10^4$ IU of NATtrol Human HCV (ZeptoMetrix) were spiked into serum and fresh blood (final concentration 0.9X).
2. All mixed in throat swab: 1 μL (OD600 = 1.0) of *C. albicans*, 500 copies of *Neisseria gonorrhoeae* positive control (ZeptoMetrix) and 1,500 copies of AcroMetrix™ HPV-16 Genotype Control (Thermo Scientific) were spiked into throat swabs (final concentration 0.9X).

Clinical specimens:

300 μL of clinical samples with sexually transmitted pathogens were used as samples (Sample IDs: S000xxx636, S000xxx647, S000xxx663, S000xxx073, S000xxx674, S000xxx019, S000xxx626, S000xxx627, S000xxx620, S000xxx623, S000xxx623, S000xxx110, S000xxx679, S000xxx332, S000xxx859, S000xxx783)

Instruments

1. TANBead® Nucleic Acid Extractor: Maelstrom™ 4810 series (M4810)
2. CFX96 Real-time PCR Detection System (Bio-Rad)

Reagent

1. TANBead® Nucleic Acid Extraction Kits:
   665 series kit (665), 66P series kit (66P)
2. Pathogen Detection kits:
   (1) DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit (SolGent)
   (2) DiaPlexQ™ STI 12 Detection Kit (SolGent)
   (3) KingCar EasyQuant HCV (KingCar)

qPCR primers and probes

Primer/probe ID	Sequence (5' to 3')
HPV16	
A-HPV16-F	CAG xxx GAA
A-HPV16-R	CCA xxx CAT
A-HPV16-P	TGT xxx CAT
HPV18	
HPV-18 E1-F	CAT xxx AGC
HPV-18 E1-R	ACT xxx ACC
HPV-18 E1-P	AGA xxx ATG
HIV	
HIV1_probe	ACA xxx ACT
HIV1qF525	TCA xxx TGA
HIV1qR599	AGG xxx CCA
λT	ATGC xxx ACT
***Staphylococcus* sp.**	
*Staphylococcus aureus*_F	AAAT xxx ACA
*Staphylococcus aureus*_R	GAA xxx GTA
*Staphylococcus aureus*_P	AAT xxx TTT
***Listeria* sp.**	
Listeria spp._F	GTTA xxx TGG
Listeria spp._R	TTT xxx TAA
Listeria spp._P	ATG xxx AAT
***Salmonella* sp.**	
*Salmonella*_invA_F	CAA xxx TGT
*Salmonella*_invA_R	CCC xxx ATT
*Salmonella*_invA_P	CTC xxx ACC
Pseudomonas aeruginosa	
*Pseudomonas aeruginosa*_F	GGC xxx GTC
*Pseudomonas aeruginosa*_R	TGG xxx TCT
*Pseudomonas aeruginosa*_P	TGC xxx ACA
C. albicans	
LH1	AGC xxx TCT
LH2	TTG xxx ATG
SC1F	CGG xxx CAC
SC1R	AGT xxx TGC

(F, forward primer; R, reverse primer; P, probe)

Automated nucleic acid extraction

1. Add 300 µL sample and 10 µL proteinase K (PK) to well #1 / #7 of Auto Plate.
2. Then process according to IFU and select the program for nucleic acid extraction (see below for program settings).

Program

665-rapid program of 665 kit on Maelstrom 4810 series

Program Name: 665-rapid							
Temp1	Temp2						
Off	Off						
Well	Name	Volume	Action	Mixing	Collect		
1	LB	900	Rev. U/D	Low	Low		
2	WB1	800	For.	Low	Low		
3	WB2	800	For.	Low	Low		
4	WB2	800	For.	Low	Low		
5	MB	800	For.	Low	Low		
6	EB	150	For.	Low	Low		
Step	Well	Temp. (°C)	Mix time (min)	Mix Speed (rpm)	Collect time (min)	Vapor time (min)	Pause
1	5		0	3000	0.5	0	Off
2	1	60	8	3000	0.5	0	Off
3	2		1	3000	0.5	0	Off
4	3		1	3000	0.5	0	Off
5	4		1	3000	0.5	5	Off
6	6	45	5	3000	0.5	0	Off
7	3		0.2	3000	0	0	Off

66P program of 66P kit on Maelstrom 4810 series

Program Name: 66P							
Temp1	Temp2						
100	100						
Well	Name	Volume	Action	Mixing	Collect		
1	LB (with GB)	900	For. U/D	Low	Low		
2	WB1	800	For.	Low	Low		
3	WB1	800	For.	Low	Low		
4	MB	800	For.	Low	Low		
5	–	–	–	–	–		
6	EB	80	For.	Low	Low		
Step	Well	Temp (°C)	Mix time (min)	Mix Speed (rpm)	Collect time (min)	Vapor time (min)	Pause
1	4		0	0	0.1	0	Off
2	1		0.1	3000	0	0	Off
3	1	100	3.5	3000	0.2	0	Off
4	2		0.1	3000	0	0	Off
5	1		0.1	3000	0.2	5	Off
6	2		0.5	3000	0.4	0	Off
7	3		1	3000	0.3	0	Off
8	6	100	0.8	2500	0.4	0	Off

Results

The extraction performance of 665 kit and 66P kit was compared using pure samples, complex samples, and clinical specimens. First, pure samples refer to a single pathogen spiked into the UTM. Nucleic acid extraction performance of 665 and 66P was evaluated using qPCR. In pure samples, 66P demonstrated better extraction performance than 665, improving Ct values by approximately 0-4.0. In virus samples, there was generally little difference in the extraction performance between 66P and 665. However, in qPCR analysis, the extraction efficiency of HPV16 using 66P was detected 2-3 cycles earlier than using 665. In bacterial samples, 66P exhibited superior nucleic acid extraction efficiency for both Gram-negative and Gram-positive bacteria. A more pronounced effect was observed in Gram-negative bacteria, resulting in an improvement of approximately 3.0-4.0 Ct values. Moreover, 66P also had a significant enhancement in the extraction efficiency of fungal samples compared to 665 **(Fig.1)**.

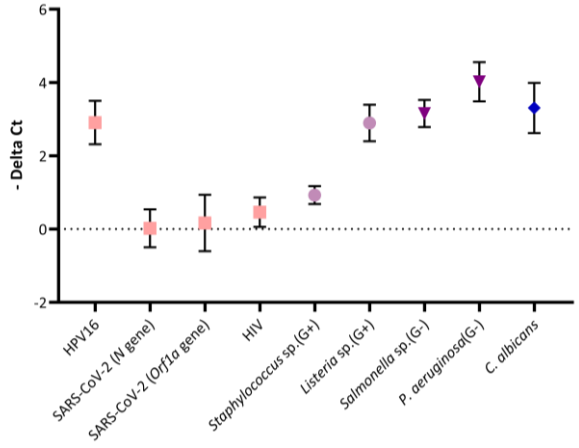

Figure 1. Performance comparison of 665 and 66P for nucleic acid extraction from pure samples. Nucleic acids were extracted from pure samples using 665 and 66P kits on M4810, followed by qPCR analysis. Delta Ct value was calculated by subtracting the Ct value of 665 kit qPCR result from the Ct value of 66P kit qPCR result. Samples not detectable by qPCR were assigned a Ct value of 50 for calculation. (-Delta Ct= - [(qPCR results of 66P kit) – (qPCR results of 665 kit)])

Second, complex samples involve the mixture of multiple pathogens in samples with complex background, including fresh whole blood, serum, and throat swabs. Generally, 665 was more efficient than 66P in extracting viral and bacterial nucleic acids from blood and serum. This is because 665 contains an additional wash buffer that helps eliminate the negative impacts caused by complex background of the samples. On the contrary, 66P had higher nucleic acid extraction efficiency for fungi in blood and serum samples. In throat swab samples, 66P exhibited better nucleic acid extraction performance for viral, bacterial, and fungal pathogen. The results demonstrated significant differences in extraction efficiency between different sample types, suggesting that the complex background of the samples had an impact on extraction performance **(Fig.2)**.

Third, STI clinical specimens were collected from patients infected with sexually transmitted pathogens. In most STI clinical specimens, 66P outperformed 665 for nucleic acid extraction of viral, bacterial, and fungal pathogens with 1.0-3.0 lower Ct values. Nonetheless, in a minority of samples, the extraction performance of 66P was comparable to that of 665, with a difference of less than 0.5 Ct. Interestingly, in certain specimens, the nucleic acid extraction of *C. trachomatis, U. urealyticum,* and *C. albicans* could only be achieved using 66P **(Fig. 3)**.

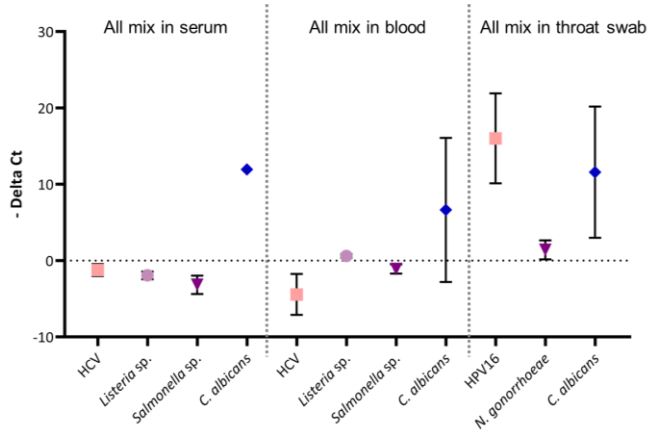

Figure 2. Performance comparison of 665 and 66P for nucleic acid extraction from complex samples. Complex samples included serum, blood, and throat swab spiked with pathogens (HCV, *Listeria* sp., *Salmonella* sp., and *C. albicans*). Nucleic acids were extracted from complex samples using 665 and 66P kits on M4810, followed by qPCR analysis. Delta Ct value was calculated by subtracting the Ct value of 665 kit qPCR result from the Ct value of 66P kit qPCR result. Samples not detectable by qPCR were assigned a Ct value of 50 for calculation. Different samples were separated by vertical dashed lines. (-Delta Ct= - [(qPCR results of 66P kit) – (qPCR results of 665 kit)])

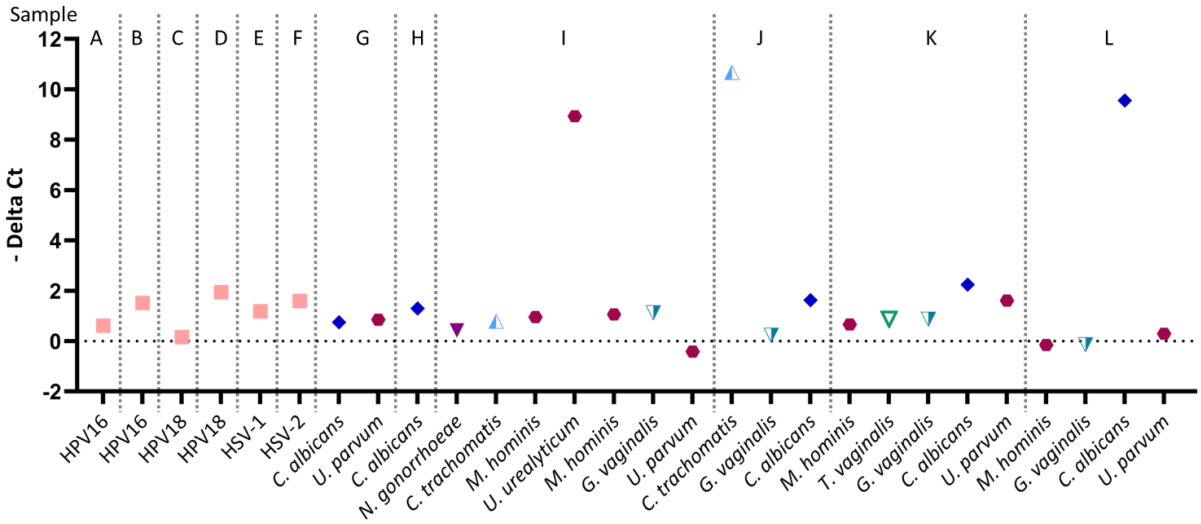

Figure 3. Performance comparison of 665 and 66P for nucleic acid extraction from clinical specimens. Clinical specimens included swabs (samples E–H, I, and L), ThinPrep® liquid (samples A–D), and urine (samples J and K). Nucleic acids were extracted from clinical specimens using 665 and 66P kits on M4810, followed by qPCR analysis to detect STI pathogens. Delta Ct value was calculated by subtracting the Ct value of 665 kit qPCR result from the Ct value of 66P kit qPCR result. Samples not detectable by qPCR were assigned a Ct value of 50 for calculation. Different samples were separated by vertical dashed lines. (-Delta Ct= - [(qPCR results of 66P kit) − (qPCR results of 665 kit)])

Conclusion

Based on our findings, TANBead® Extraction Kit 66P series is capable of efficiently purifying high-quality DNA or RNA from various samples, including UTM, whole blood, serum, and urine. Additionally, 66P contains glass beads in the lysis buffer and undergoes extraction at high temperature, which can more effectively lyse pathogens and facilitate the extraction of nucleic acids from viruses, bacteria, and fungi. When comparing the extraction performance of 665 and 66P, 66P showed better extraction efficiency in samples with less complex background, whereas 665 is suitable for more complex samples such as blood and serum. Notably, the extraction process of 66P can be completed in approximately 20 minutes without any sample pretreatment. In summary, considering the time saving and efficiency, 66P is recommended for the extraction of pathogenic nucleic acids from a wide range of sample types, particularly those that may contain multiple unknown pathogens.

Ordering information

Product Name	Test	Instrument	Reference No.	Ordering No. (IVD)	Ordering No. (RUO)
TANBead® Universal Pathogen Auto Plate	96	M8 / M4800 series	M66PA46	-	301712
TANBead® Universal Pathogen Auto Tube	96	M8 / M4800 series	M66PS46	-	301713