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Nucleic acid extraction without electrical equipment via magnetic nanoparticles in Pasteur pipettes for pathogen detection

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ABSTRACT

The outbreak of COVID-19 makes epidemic prevention and control become a growing global concern. Nucleic acid amplification testing (NAAT) can realize early and rapid detection of targets, thus it is considered as an ideal approach for detecting pathogens of severe acute infectious diseases. Rapid acquisition of high-quality target nucleic acid is the prerequisite to ensure the efficiency and accuracy of NAAT. Herein, we proposed a simple system in which magnetic nanoparticles (MNPs) based nucleic acid extraction was carried out in a plastic Pasteur pipette. Different from traditional approaches, this proposed system could be finished in 15 min without the supports of any electrical instruments. Furthermore, this system was superior to traditional MNPs based extraction methods in the aspects of rapid extraction and enhancing the sensitivity of a NAAT method, accelerated denaturation bubbles mediated strand exchange amplification (ASEA), to the pathogens from various artificial samples. Finally, this Pasteur pipette system was utilized for pathogen detection in actual samples of throat swabs, cervical swabs and gastric mucosa, the diagnosis results of which were identical with that provided by hospital. This rapid, easy-performing and efficiency extraction method ensures the applications of the NAAT in pathogen detection in regions with restricted resources.

1. Introduction

Due to the characteristics of earlier detection, higher sensitivity and specificity than traditional antibody-based methods, NAAT has become one of main approaches for pathogen detection in clinical diagnosis and food safety inspection at present, which has played an important role in SARS-CoV-2 detection since 2020 [1,2]. In order to further shorten time consumption of NAAT, as well as improve its sensitivity and specificity, variety of amplification technologies have been proposed since PCR technology was first reported, e.g. LAMP, NASBA, RCA and RPA [3–6]. In addition to progressing amplification technologies, the obtaining of high-quality nucleic acid is another key factor of successfully applying NAAT on pathogen detection since nucleic acid extraction is normally the first step [7,8].

Nucleic acid extraction generally involves the procedures of cell (or virion) lysis, nucleic acid isolation and purification [9]. Chemical lysis is the most widely used approach to release nucleic acid into the buffer, which are the basic of most commercial kits [10]. However, some of the chemical agents introduced for disintegrating cell (or virion) structure, such as guanidinium and SDS, would strongly inhibit downstream amplification [11], therefore efficient isolation and purification of nucleic acid from lysis buffer are critical to ensure the efficiency and sensitivity of NAAT. As reported, solution-based extraction and column-based extraction are commonly used methods for nucleic acid isolation and purification currently [12]. Nevertheless, these methods normally involve multiple time-consuming sample handling steps, which require the support of trained staff and bulky sophisticated instruments [13]. Therefore, the applications of these methods are restricted in primary medical units lacking of funding or grass-root areas [14]. In order to simplify to nucleic extraction process, researchers proposed alternative approaches that could rapidly disintegrate cells (or virions), e.g. thermal lysis and corona discharge lysis methods, in which the samples are directly added to reaction system after briefly treated by a heating block or corona treater [15,16]. However, these methods that pursuing ultrafast and ultrasimple normally include no nucleic acid

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Abbreviation list

NAAT	Nucleic acid amplification testing
MNPs	Magnetic nanoparticles
POCT	Point of care testing
HPV-16	Human papilloma virus type 16
COVID-19	Corona virus disease 2019
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
ASEA	Accelerated denaturation bubbles mediated strand exchange amplification

purification or enrichment processes, leads to the low sensitivity of NAATs. Thus, they could be hardly applied on the samples rich in organic or contain the ingredients strongly inhibiting amplification, such as tissue and serum [17]. In addition, high temperature and high voltage electrical involved in these methods might injure the operators. Due to the problems of current extraction methods mentioned above, the development of simple nucleic acid extraction methods that could efficiently recover and purify target nucleic acids of pathogens from various samples with minimum equipment is meaningful for both scientific research, epidemic prevention and control, as well as food safety inspection.

Magnetic nanoparticles (MNPs) are applied for nucleic acid isolation and purification in recent years, which could bind nucleic acid by the free chemical groups decorated on the surface [18–20]. Normally, since the Fe_3O_4 core of MNPs allows them to be gathered by magnets, as well as the nucleic acid binding on them could be rapidly eluted by aqua, this heterogeneous extraction process is greatly simplified and have the potential to be utilized for the development of "universal" nucleic acid extraction methods for pathogen detection in wide range of samples [21,22]. However, the current MNPs based methods include frequency liquid handling steps by pipette, which may lead to the waste of MNPs and samples, or the remaining of large amount of buffer when discarding supernatant. Therefore, these methods require vortex mixer and centrifuge to assist in the dispersion and collection of MNPs, as well as dry bath to evaporate residual buffer, which undoubtedly complicates the extraction process.

In this work, we described an approach of conducting MNPs based nucleic acid extraction procedure in an ordinary plastic Pasteur pipette with no vortex mixer, centrifuge, or dry bath, and applied this approach to extract nucleic acid of various pathogens, including RNA virus, DNA virus, gram-negative bacteria, and gram-positive bacteria from a broad range of samples. The MNPs of a commercial kit that adopts traditional MNPs based nucleic acid extraction method were employed to extract nucleic acid of pathogens from same samples via both Pasteur pipette system and this commercial kit. The extracted nucleic acid was directly used as template for accelerated denaturation bubbles mediated strand exchange amplification (ASEA), a rapid and convenient amplification method established by us previously [23,24]. Extraction efficiency of our approach was evaluated by comparing the detection time and sensitivity of ASEA to the target nucleic acid extracted by these two methods. The objective is to provide a simple, rapid, and easy operating nucleic acid extraction method for pathogen detection of various samples in the regions with limited resources.

2. Materials and methods

2.1. Materials and reagents

Actual samples including throat swabs collected from the patients diagnosed with *Mycoplasma pneumoniae* infection, human papilloma virus type 16 (HPV-16) positive cervical swabs, as well as gastric mucosa of the patients suffering severe stomachache with suspected *Helicobacter pylori* infection were provided by the Affiliated Hospital of Qingdao University. All the samples were immediately stored at −20 °C after collection for subsequently use. *Streptococcus aureus* (ATCC 25923), *Salmonella typhimurium* strains (ATCC 14028) were provided by Navid Biotechnology Co., Ltd (Qingdao, China). SARS-CoV-2 pseudovirus was purchased from Fubio Biological Technology Co., Ltd (Shanghai, China). Guanidine hydrochloride, guanidine isothiocyanate, proteinase K, ethanol, isopropanol, SDS, TritonX-100, Tween-20 and pig serum were purchased from Sangon Biotech Co., Ltd (Shanghai, China). All the other chemicals and reagents were of analytical grade.

2.2. Preparation of artificial samples

Artificial SARS-CoV-2 positive throat swabs were prepared by dropping 100 μL SARS-Cov-2 pseudovirus suspension on the swabs newly swabbing volunteers' throat. Artificial *S. aureus* infected serum was prepared by mixing 100 μL *S. aureus* suspension into 900 μL pig serum. Artificial *S. typhimurium* contaminated milk was prepared by mixing 100 μL *S. typhimurium* suspension into 900 μL commercial milk. Artificial *S. typhimurium* contaminated pork was prepared by immersing 10 mg commercial pork into 1 mL *S. typhimurium* suspension for 15 min, which were then grinded into tissue homogenate. Moreover, artificial infected serum and contaminated foods prepared with corresponding bacterial fluids with final concentration of 1.0×10^6, 1.0×10^5, 1.0×10^4, 1.0×10^3, 1.0×10^2, 1.0×10^1 and 1.0×10^0 CFU/mL, as well as artificial throat swabs containing SARS-CoV-2 pseudovirus suspension with the final RNA concentration of 1.0×10^7, 1.0×10^6, 1.0×10^5, 1.0×10^4, 1.0×10^3, 1.0×10^2 and 1.0×10^1 copies/mL were utilized as the targets to assess the sensitivity of ASEA to the pathogens in various types of samples via nucleic acid extracted by this proposed method and the commercial kit.

2.3. Nucleic acid extraction in a Pasteur pipette

Lysis buffer and washing buffer were prepared by referencing the reports of Dignan et al., Liu et al. and Wang et al. with some modifications [25–27]. Specifically, 200 μL samples and 30 μL MNPs suspension were added into a mixture composed of 300 μL lysis buffer (50 mM Tris, 10 mM EDTA, 0.1 M NaCl, 4 M guanidine isothiocyanate, 0.1% SDS [w/v], 2 mg/mL proteinase K, 5% Tween-20 [v/v], and 3% TritonX-100 [v/v], pH 8.0) and 300 μL isopropanol in a 1.5 mL centrifuge tube. Then the mixture was gently sucked up and down for several times by a Pasteur pipette and placed at room temperature for 5 min. Subsequently, the liquid in the Pasteur pipette was removed after collecting MNPs on the wall of Pasteur pipette by a magnet (Fig. 1A). The washing step was performed through pipetting up and down for several times in 400 μL washing buffer (5 M guanidine hydrochloride, 20 mM EDTA, 20 mM Tris and 50% isopropanol [v/v], pH 8.0) and 600 μL ethanol solution (80%, w/w) successively to remove contaminants and amplification inhibitors. Then the MNPs were dried in Pasteur pipette by pipetting air up and down several times and placed at room temperature for 1 min (Fig. 1B). Last, the nucleic acid absorbed on MNPs was eluted by immersing MNPs in 30 μL nuclease free water for 3 min at room temperature (Fig. 1C). The nuclease free water was subsequently collected and performed as templates for amplification (Fig. 1D). The whole extraction process could be finished in 15 min without any electrical instruments, significantly shorter than that suggested in the manufacturer's instruction of the commercial kit. Briefly, the traditional method including 10 min of lysis and binding process, 1 min of washing process (repeated three times), 10 min of drying process at 56 °C, and 10 min of elution process at 56 °C. During these processes, vortex mixer was employed for the thorough mixing of samples, buffers and MNPs, while dry bath and centrifuge were employed for assisting in nucleic acid collection and purification. The processes of Pasteur pipette system and traditional MNPs based extraction methods of the commercial kit were shown in Table 1.

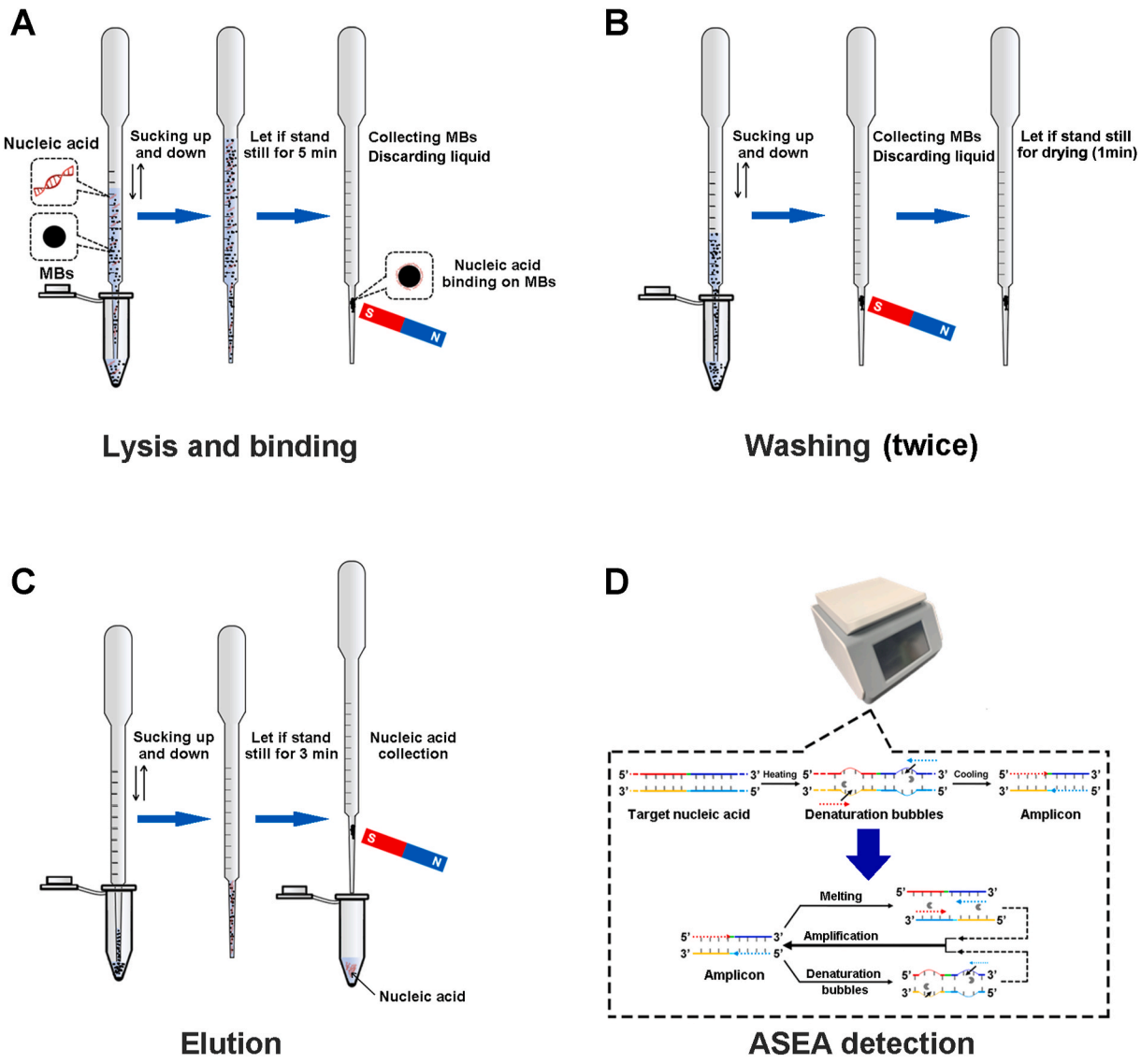

Fig. 1. Nucleic acid extraction in Pasteur pipette system and ASEA detection. Schematic illustration of (A) lysis and binding step, (B) washing step and (C) elution step of nucleic acid extraction in Pasteur pipette system. (D) The mechanism of ASEA reaction.

Table 1
Overview of process and time consumption of Pasteur pipette method and the commercial kit adopting traditional MNPs based nucleic acid extraction method.

Processes	Time consumption		Electrical equipment involved	
	Pasteur pipette	Commercial kit	Pasteur pipette	Commercial kit
Lysis and binding	5 min	10 min	No	Vortex mixer
Washing	2 min	3 min	No	Vortex mixer
Drying	1 min ([a]RT)	10 min (56 °C)	No	Dry bath and Centrifuge
Elution	3 min (RT)	10 min (56 °C)	No	Dry bath and Centrifuge
Total	**15 min**	**35 min**	**0**	**3**

[a] RT represented room temperature.

2.4. Primer design

Primers specifical to *S. aureus* 16S rDNA, *S. typhimurium fimbriae Y* (*fimY*) gene, *M. pneumoniae* 16S rDNA, SARS-CoV-2 *orf1ab* gene, HPV-16 *L1* gene and *H. pylori* 16S rDNA were designed and optimized by NCBI primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast) and NUPACK software (http://www.nupack.org/), and synthesized by Sangon Biotech (Shanghai, China). The sequences of the primers used in this work were shown in Table 2.

2.5. ASEA reaction

ASEA reaction was performed in 20 μL amplification mixture containing 2 μL templates, 6 μL forward (F) and reverse (R) primers (3.0×10^{-6} M), 2 μL ISO Buffer, 0.5 μL Eva Green, 0.5 μL polyethylene glycol (PEG), 0.5 μL Bst 2.0 WarmStart DNA polymerase and 1.6 μL dNTPs. The reaction procedure included 40 rapid cycles of 74 °C for 1 s, 61 °C for 1 s for amplification [28]. During the thermal cycling process, the heating rate and cooling rate were 5 °C/s and 3 °C/s, respectively. For RNA templates, a reverse transcription step, i.e. 55 °C for 5 min, was added in prior to the rapid thermal cycling in reaction procedure.

2.6. Pathogen detection of actual samples

Preservation fluid used for throat swabs and cervical swabs storage, as well as homogenate of gastric mucosa specimens were utilized for

Table 2
Sequences of primers used in this work.

Name	Sequence (5′-3′)
S. aureus 16S rDNA ([a]NR_118997.2)	
Primer F	TGGTTCAAAAGTGAAAGACGG
Primer R	CCAACTAGCTAATGCAGCG
***S. typhimurium fimY* gene ([a]M90677.1)**	
Primer F	ATCAGAGGCTTTTTATGCCG
Primer R	GAGGTCTTTGCGTTTACTTACA
***M. pneumoniae* 16S rDNA ([a]NR_041751.1)**	
Primer F	GGCGAAGGCGAAAACTTA
Primer R	CAAGCCTAAGCGTCAGTA
SARS-CoV-2 *orf1ab* gene ([a]NC_045512.2)	
Primer F	AACACAGTCTGTACCGTC
Primer R	ACCTTTCCACATACCGCA
HPV-16 *L1* gene ([a]FJ797057.1)	
Primer F	TTTGTTACTGTTGTTGATACTAC
Primer R	GAAAAATAAACTGTAAATCATATTC
***H. pylori* 16S rDNA ([a]MT477178.1)**	
Primer F	ATCGCTAAGAGATCAGCCTA
Primer R	TAGCCTTGGTAAGCCATT

[a] GenBank accession number.

nucleic acid extraction via both Pasteur pipette system and the commercial kit. The extracted nucleic acid was then used as template for pathogen detection by ASEA. Nucleic acid extraction efficiency of Pasteur pipette system on actual samples was assessed by comparing the time consumption with the commercial kit.

3. Results and discussion

3.1. Evaluation of Pasteur pipette system applicability for various samples

The nucleic acid extracts of artificial SARS-CoV-2 positive throat swabs (prepared with 10^7 copies/mL SARS-CoV-2 pseudovirus suspension), infected serum (prepared with 10^5 CFU/mL *S. aureus* suspension) and contaminated food samples (prepared with 10^5 CFU/mL *S. typhimurium*) obtained by Pasteur pipette system and the commercial kit were applied as target for ASEA to assess the applicability of Pasteur pipette system for NAAT. As shown in Fig. 2, accumulation of fluorescent signal was detected in all the reactions with the nucleic acid extracted by both Pasteur pipette system and the commercial kit as template. Moreover, the Ct values of all kinds of samples obtained after the extraction via Pasteur pipette system were obviously lower than those treated with the commercial kit. Specifically, the average Ct value obtained from extractions of Pasteur pipette system were 12.41 for throat swab specimens, 20.27 for infected serum specimens, 19.20 for contaminated milk samples and 20.60 for contaminated pork samples, while the average Ct values of the samples obtained from extractions of the commercial kit were 17.51, 23.28, 22.43 and 23.49, respectively. We supposed this phenomenon might due to partial cells (or virions) were failed to be completely disintegrated during lysis step, therefore the guanidine hydrochloride in washing buffer of Pasteur pipette system would continue hydrolyzing histone or capsid, which allow more nucleic acid releasing to the buffer in washing step and increase nucleic acid recovery rate. Besides, the optimized lysis buffer and washing buffer utilized in this

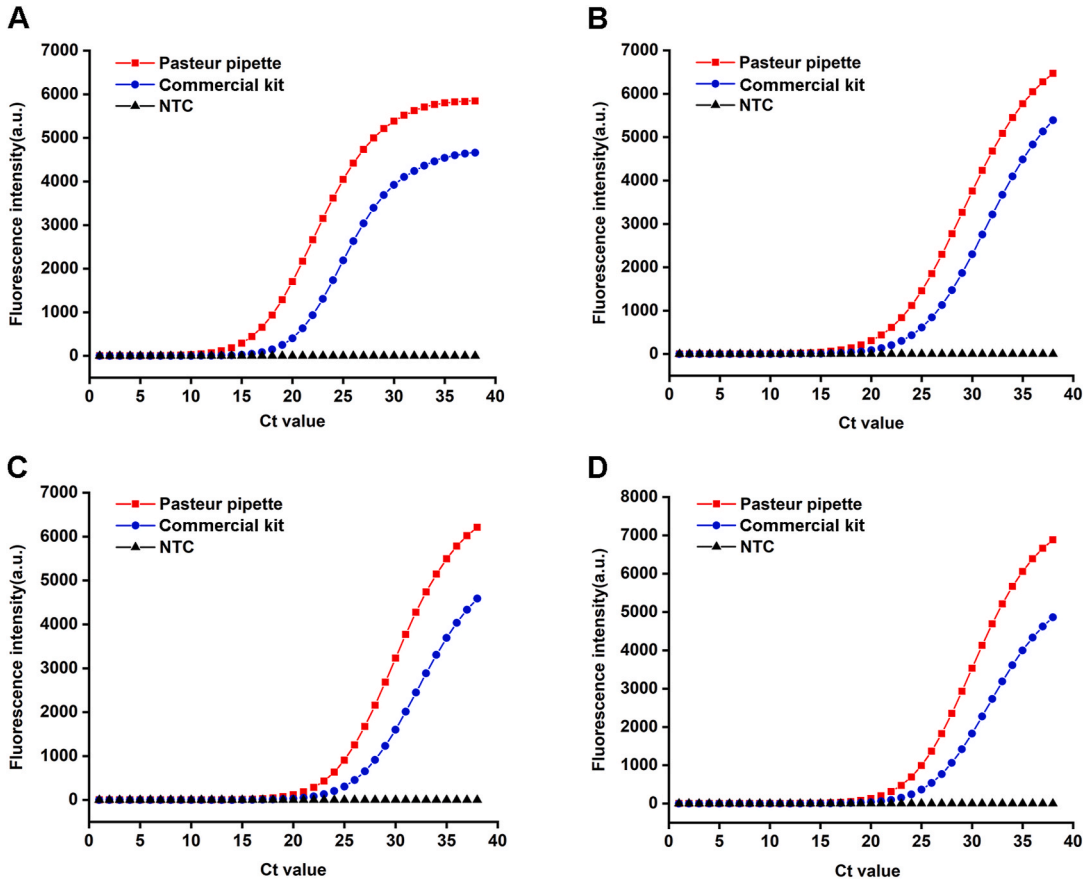

Fig. 2. Application of Pasteur pipette system on nucleic acid extraction from bacterial suspension and different types of artificial samples. Fluorescence curves of amplification reaction with nucleic acid prepared by Pasteur pipette system and the commercial kit from artificial (A) SARS-CoV-2 positive throat swabs, (B) *S. aureus* infected serum, (C) *S. typhimurium* contaminated milk and (D) *S. typhimurium* contaminated pork as template. NTC represented no target control.

work were also beneficial for the removal of these inhibitory component. More importantly, in the procedure of MNPs mediate nucleic acid extraction, guanidine salts, proteinase K and surfactants were employed for the lysis of cells (or virions), as well as promoting the denaturation and hydrolysis of histone or capsid [29–31], via which the nucleic acid was released to lysis buffer and then captured by MNPs. However, these ingredients could strongly inhibit the polymerase activity and amplification efficiency [32], some ingredients of the samples may also have a direct influence on amplification as well [33]. Therefore, centrifuge and dry bath were employed to ensure the removal of these inhibitors in the

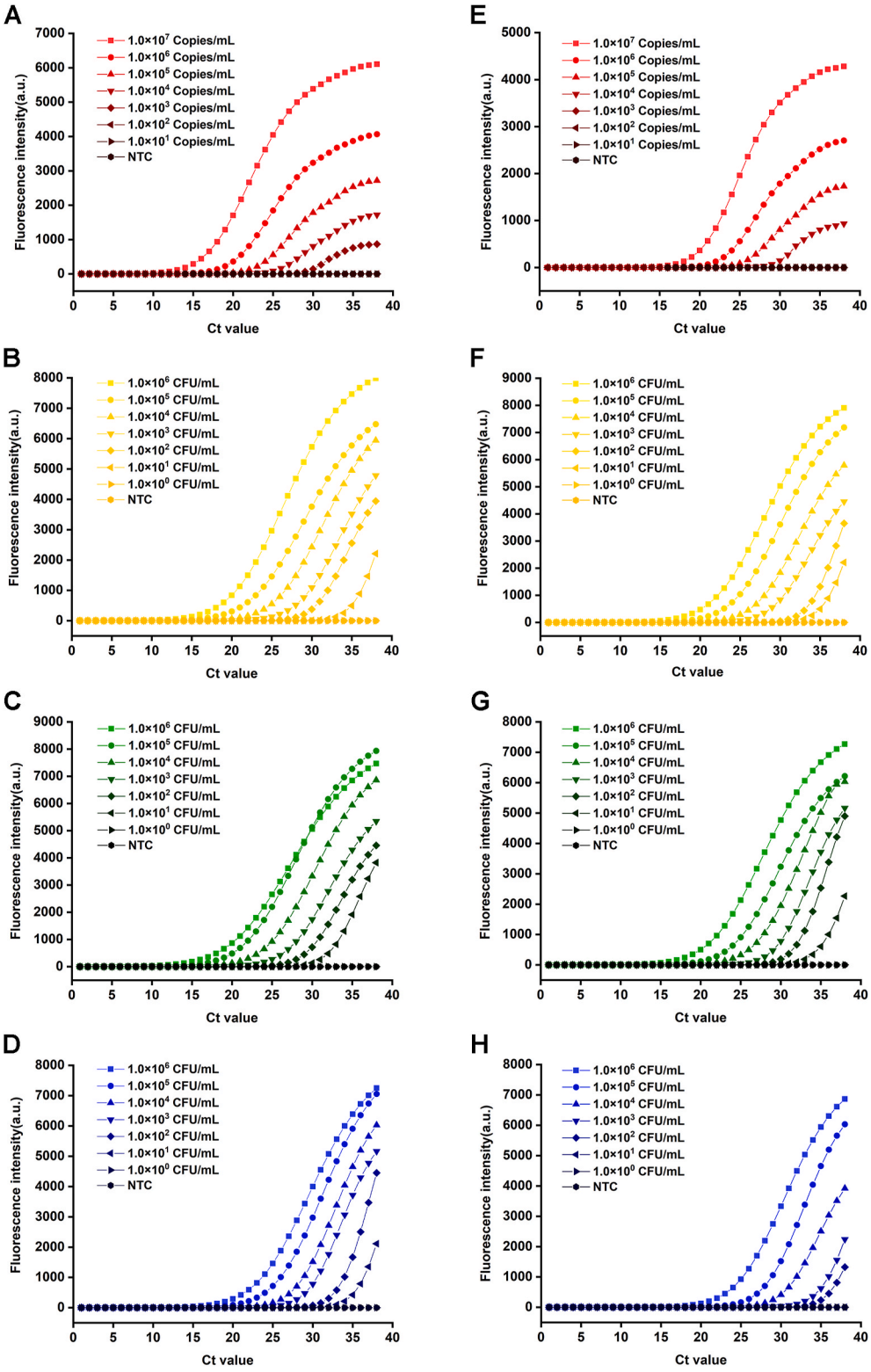

Fig. 3. Sensitivity of ASEA to the pathogens in different types of samples. Fluorescence curves of the amplification reaction with nucleic acid extracted by (A–D) Pasteur pipette system and (E–H) the commercial kit from artificial SARS-CoV-2 positive throat swabs, artificial *S. aureus* infected serum, artificial *S. typhimurium* contaminated milk, as well as artificial *S. typhimurium* contaminated pork prepared with 10-fold serial diluted target pathogens as template.

procedure of the traditional method, which is critical to improve efficiency and accuracy of NAAT. Unlike the commercial kit, Pasteur pipette system realized inhibitors removement via simply pipetting washing buffer or air up and down manually rather than electrical equipment, made the operation of this system simple and convenient. The results revealed that Pasteur pipette system had a better performance on inhibitors removal than the traditional method of the commercial kit. Additionally, no significant fluorescent signal accumulated was detected in the reaction with the extractions from no target controls, suggested the treatment in Pasteur pipette system would not cause nonspecific amplification. This phenomenon demonstrated hardly any cross contamination was occurred during extraction progress. In sum, Pasteur pipette system is applicable for various specimens and superior to the traditional MNPs based extraction methods applied by many commercial kits in the aspects of efficiency and convenience.

3.2. Sensitivity of ASEA to nucleic acid extracted by Pasteur pipette system from various samples

NAAT sensitivity to target nucleic acid of pathogen in samples is another parameter for the assessment of nucleic acid extraction methods, as excellent nucleic acid extraction methods should be able to gather and concentrate nucleic acid effectively [34], especially from the samples containing trace amounts of target pathogens, such as the throat swabs of SARS-CoV-2 asymptomatic carriers. Hence, we also determined the sensitivity of ASEA to pathogens in various samples after nucleic acid extraction with Pasteur pipette system and the commercial kit adopting traditional MNPs based method, respectively. The results showed that the ASEA sensitivity obtained by Pasteur pipette system was similar or higher than that obtained by the commercial kit. Specifically, ASEA could successfully detect 1.0×10^3 copies/mL SARS-CoV-2 pseudovirus in throat swabs samples and 1.0×10^1 CFU/mL *S. typhimurium* in pork samples after the extraction by Pasteur pipette system (Fig.3A and D), which was failed to be achieved after the extraction by the commercial kit (Fig. 3E and H). Although the sensitivities of ASEA to *S. aureus* in serum samples and *S. typhimurium* in milk were comparable after the extraction by Pasteur pipette system and the commercial kit (Fig. 3B, C, F and G), coincide with the results of nucleic acid extraction efficiency evaluation, the nucleic acid extracted by Pasteur pipette system could be detected earlier, exhibited as the obviously lower Ct values. Despite of that the short reverse transcription step integrated in ASEA procedure led to less DNA templates involved in amplification reaction, which cause lower sensitivity of ASEA on SARS-CoV-2 than some reported methods based on PCR [35,36], these results still demonstrated Pasteur pipette system was superior to traditional MNPs based extraction method in the aspect of increasing the sensitivity of ASEA to SARS-CoV-2 pseudovirus in throat swab, as well as other target pathogens in the samples of serum, milk and pork. Moreover, the Ct values of the nucleic acid extracted by Pasteur pipette system exhibited a significant linear relationship with the logarithm of target pathogens' concentration in all types of samples ($R^2 > 0.99$), which were similar with that of the nucleic acid prepared by the commercial kit, demonstrated Pasteur pipette system possessed stability yield rate in the experimental concentration range.

3.3. Validation of Pasteur pipette system applicability on actual samples

The acquirement of high-quality nucleic acid is one of the challenges in the application of NAAT on actual samples, since these samples are normally more complex in composition than the artificial ones, which would make nucleic acid purification difficult [37], especially the clinical samples. Moreover, some of the ingredients may strongly inhibit nucleic acid extraction or amplification, and affect the efficiency and accuracy of NAAT [38]. In this work, actual samples including 8 throat swabs samples, 12 cervical swabs samples and 13 gastric mucosa samples were employed to evaluate the performance of Pasteur pipette system on practical application. As shown in Table 3, *M. pneumoniae* 16s rDNA was successfully detected by ASEA with the extracts of each throat swab prepared by Pasteur pipette system as templates, indicated *M. pneumoniae* nucleic acid was successfully extracted. This result was consistent with the diagnosis result of serological test provided by hospital, that is, all the swab donors were infected with this pathogen. Although the composition of cervical swabs collection is normally more complex than that of throat swabs, since some of these swabs contain blood and cervical mucus, same as throat swabs, pathogens (HPV-16) were also detected in each cervical swab after nucleic acid extraction by Pasteur pipette system, which was coincide with the diagnosis result of hospital, demonstrated Pasteur pipette system was efficient in removing the components that inhibit amplification existed in blood or cervical mucus.

Besides swabs, Pasteur pipette system was also applicable for nucleic acid extraction of gastric mucosa samples, the ASEA result of which showed that *H. pylori* genome was detected from gastric mucosa samples of the patients diagnosed as *H. pylori* infection by gastric biopsy, while no significant fluorescent signal accumulation was detected in the samples from *H. pylori* negative donors (−). Moreover, the nucleic acid extracts of gastric mucosa samples from the patients suffering severe *H. pylori* infection, which was confirmed as strong positive (+++) by gastric biopsy, possessed obviously lower Ct value than those confirm as medium positive (++) or weak positive (+), illustrated that the strong positive samples had higher *H. pylori* content according to ASEA result (Table 3). Due to the ASEA result on *H. pylori* content was in consistence with that of gastric biopsy provided by hospital, it could be concluded that Pasteur pipette system could not only successfully extract nucleic acid from tissue samples, but also was stability in yield rate.

In addition to Pasteur pipette system, the commercial kit adopting traditional MNPs based method was also employed to extract nucleic acid from above samples. As shown in Table 3, similar with Pasteur pipette system, by using the nucleic acid extracted via the commercial kit as templates, the ASEA diagnosis results of these samples were also identical with that provided by hospital, demonstrated this commercial kit is applicable to these actual samples. However, all the Ct values of the reaction using nucleic acid extracted by the commercial kit as templates were obviously higher than that using nucleic acid extracted by Pasteur pipette system from same samples as templates, demonstrated the ASEA was more sensitive to the genome extracted by Pasteur pipette system. Moreover, the extraction process of Pasteur pipette system required less time consumption than the traditional MB based method of commercial kit, and was free of heating devices or centrifuge equipment. Thus, the Pasteur pipette system was desirable to be utilized in diagnosis of pathogens from actual samples, and would be of great beneficial to the POCT during the outbreak of epidemic.

4. Conclusion

In this present work, we successfully established a novel Pasteur pipette system as a universal approach for nucleic acid extraction of pathogen via MNPs from various samples including swabs, serum, milk and pork, the time consumption of the which was approximate 15 min. More importantly, differ from most of widely used nucleic acid extraction methods including those based on MNPs, this proposed approach involved no toxic organic reagents or electrical equipment, makes it rapid and convenient. Compared with a widely used commercial kit that adopts traditional MNPs based extraction method, the extraction products of Pasteur pipette system could be detected earlier by ASEA, the limit of detection of which was lower by using the nucleic acid extraction obtained from Pasteur pipette system as well, demonstrated Pasteur pipette system was more efficient on nucleic acid purification and concentration than traditional MNPs based extraction method. Moreover, the detection results by ASEA with nucleic acid extracted by Pasteur pipette system as templates were identical with that provided by hospital. Since Pasteur pipette system exhibited more excellent

Table 3
ASEA results of the actual samples prepared by Pasteur pipette system and the commercial kit adopting traditional MNPs based nucleic acid extraction method.

Actual sample	Target pathogen	Patient number	Ct value		Hospital diagnosis results
			Pasteur pipette	Commercial kit	
Throat swab	*M. pneumoniae*	1	21.80	24.39	Positive
		2	21.53	24.29	Positive
		3	20.74	23.15	Positive
		4	11.42	14.16	Positive
		5	14.48	15.19	Positive
		6	10.78	13.88	Positive
		7	11.63	14.33	Positive
		8	13.29	15.03	Positive
		[a]NTC	[b]NoCt	NoCt	[c]N/A
Cervical swab	HPV-16	9	24.94	28.54	Positive
		10	27.90	35.21	Positive
		11	26.14	29.56	Positive
		12	26.69	30.40	Positive
		13	28.70	30.32	Positive
		14	27.90	28.09	Positive
		15	25.61	26.45	Positive
		16	26.35	28.40	Positive
		17	27.90	28.49	Positive
		18	27.92	28.76	Positive
		19	28.08	30.39	Positive
		20	20.5	21.23	Positive
		NTC	NoCt	NoCt	N/A
Gastric mucosa	*H. pylori*	21	19.93	21.52	+++
		22	19.21	20.51	+++
		23	20.42	21.56	+++
		24	19.84	20.54	+++
		25	20.59	22.10	++
		26	21.66	23.15	++
		27	21.88	22.08	++
		28	28.11	29.12	+
		29	25.34	26.33	+
		30	23.44	25.44	+
		31	NoCt	NoCt	-
		32	NoCt	NoCt	-
		33	NoCt	NoCt	-
		NTC	NoCt	NoCt	N/A

[a] NTC represented no target control.
[b] NoCt represented no detected Ct value.
[c] N/A represented not applicable.

performance than the traditional MNPs based extraction method on time consumption and convenience, this universal, rapid and convenience extraction method is particularly desirable to improve the efficiency of NAAT on pathogens diagnosis, and possesses the potential to be applied on the constructing a sample-to-answer platform with minimal equipment by integrating with colorimetric isothermal amplification technologies in near-patient use and resource-limited settings.

CRediT authorship contribution statement

Jia Kang: performed the experiments, analyzed the data; wrote the manuscript, and all authors contributed to the writing of the paper, had primary responsibility for the final content, and read and approved the final manuscript. **Yang Li:** analyzed the data; designed the experimental scheme, wrote the manuscript; and all authors contributed to the writing of the paper, had primary responsibility for the final content, and read and approved the final manuscript. **Yan Zhao:** performed the experiments; and all authors contributed to the writing of the paper, had primary responsibility for the final content, and read and approved the final manuscript. **Yanling Wang:** performed the experiments; and all authors contributed to the writing of the paper, had primary responsibility for the final content, and read and approved the final manuscript. **Cuiping Ma:** designed the experimental scheme; and all authors contributed to the writing of the paper, had primary responsibility for the final content, and read and approved the final manuscript. **Chao Shi:** designed the experimental scheme; and all authors contributed to the writing of the paper, had primary responsibility for the final content, and read and approved the final manuscript.

Declaration of competing interest

There are no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ab.2021.114445.

Ethics approval

The authorized Human Health and Ethics Committee of the Affiliated Hospital of Qingdao University approved this study and all the donors of

all clinical specimens were informed consent. In addition, all methods were carried out in accordance with the relevant guidelines and regulations.

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