Nucleic Acid Extraction and Enrichment

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Introduction

Since thermostable *Taq* DNA polymerase was discovered in 1987, nucleic acid amplification techniques have made great strides and have contributed greatly to progress in the life sciences. These techniques were introduced into the clinical laboratory where they have produced major advances in diagnostic instruments and molecular testing. In particular, there have been many innovative molecular testing developments in the field of diagnostic microbiology.

Conventional culture methods for bacterial isolation and identification are labor-intensive and time-consuming. However, they are simple and cheap and remain the gold standard. It also is relatively easy to perform antimicrobial susceptibility testing on cultured isolates, so conventional culture methods with biochemical phenotyping remain the most common procedures performed in clinical microbiology laboratories [1]. To further assist in microbial identification, nucleic acid amplification has been introduced in the clinical microbiology laboratory. Such testing was initially done for viruses, allowing detection of small amounts of viral nucleic acid quickly. Similar tests also have been applied to bacteria, especially those that are difficult to grow on routine culture media, or are slow-growing such as *Chlamydia*, *Neisseria gonorrhoeae*, and *Mycobacterium*. In addition, there are ongoing efforts to integrate these new techniques into routine clinical microbiology testing, including the diagnosis of sepsis [1].

The development of nucleic acid amplification has proceeded at an unprecedented pace and achieved higher sensitivity and specificity [2]. However, in order to obtain clinically relevant results with this new technique, the testing methodology must include several important steps. Preanalytical testing variables that can

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comprise test results include the following: (1) sample collection and preparation, (2) specimen transport and storage, (3) the stability of the nucleic acid in the samples, and (4) nucleic acid extraction [3, 4].

Nucleic acid extraction is the first and most important step for any amplification method no matter what kind of amplification is used to detect a specific pathogen [1, 5]. It is a crucial preanalytic step in the development and performance of any successful molecular diagnostic method and ensures a reliable and clinically relevant result [3, 4]. We must pay close attention to the technical progress of the nucleic acid extraction as well as to the method for amplification and detection of nucleic acids in order to obtain satisfactory results. Nucleic acid extraction consists of three major processes: isolation, purification, and concentration. Commercial extraction kits are commonly used in the clinical microbiology laboratory [2]. These kits provide the essential requirements for nucleic acid extraction. These essential requirements have been well described by Boom et al. [6, 7] as follows: (1) extraction should be simple and rapid and should show high sensitivity and specificity; (2) it is preferred that there be no requirements for specialized equipment or special knowledge and skills; (3) the final nucleic acid should be pure and easy to modify for various amplification techniques; (4) the reagents and their product should be harmless; and (5) the process of preparation should resist contamination with other specimens. If the final volume of eluate is small, detection limits are maximized. When we deal with clinical specimens, we also should consider the elimination of potential inhibitors of the DNA polymerase and the removal of pathogenicity from hazardous pathogens as well as good target recovery and establishment of the integrity of nucleic acid targets [2]. Ideally, the final target is pure nucleic acid without amplification inhibitors or contaminants such as proteins, carbohydrates, and other nucleic acids [8].

There are a few points that must be specially considered when a laboratory evaluates the use of nucleic acid extraction in the field of clinical microbiology. Usually, DNA or RNA is extracted, although sometimes both DNA and RNA are extracted, depending on the circumstances. The targets for nucleic acid extraction are diverse.

Alternatively, the microbial DNA or RNA may be amplified by the use of culture media including blood culture bottles or various clinical specimens such as sputum, stool, urine, tissue, or cerebrospinal fluid [1]. In terms of nucleic acid extraction, the same nucleic acid may be targeted, but there are different implications for the extraction procedure itself. Nucleic acid extraction from cultured bacteria is relatively simple because the isolated microbes are pure colonies that contain large numbers of organisms. However, it is important to recognize that gram-positive bacteria have thick cell walls, which makes nucleic acid extraction more difficult than it is with gram-negative bacteria, which have thinner cell walls [5]. For clinical specimens, the details of the extraction method depend on the characteristics of each specimen. It is important to remember that the goal of extraction is nucleic acid, not of humans, but of bacteria, virus, or fungus. If DNA or RNA is extracted from clinical specimens containing human cells, these extracted specimens will contain human DNA; the recovery of microbial DNA thus may be diminished by the presence of human DNA.

Nucleic Acid Extraction Techniques

Cesium Chloride/Ethidium Bromide Density Gradient Centrifugation

Since 1950, density gradient centrifugation using cesium chloride (CsCl)/ethidium bromide (EtBr) has been used for DNA extraction and has become the standard method in research laboratories [9, 10]. The basic principle of this method is utilization of the difference in density between the cesium ion and water with intercalation of EtBr; this results in good separation of various DNAs and allows the procurement of high-yield DNA [11]. For example, each DNA can be separated as independent bands because of the differences in each DNA's density in the gradient caused by the intercalation of EtBr [7]. However, there are important limitations for this method in that it requires an expensive ultracentrifuge, is labor-intensive and difficult to perform, and requires more time and EtBr is harmful [7, 8]. Consequently, this method is not suitable for use in the clinical microbiology laboratory and thus has not been used in this setting.

Phenol-Chloroform Extraction

Phenol-chloroform extraction is another method that has been widely used. The process consists of the vigorous mixing of a phenol-chloroform solution with the sample followed by centrifugation [7]. Phenol does not completely inhibit RNase activity, and this characteristic allows isolation of nucleic acid that has "dissolved" in the chloroform and phenol mixture [12]. After centrifugation, the upper (aqueous) phase containing the DNA can be separated from the lower (organic) phase containing denatured proteins, and DNA can be precipitated by adding ethanol or isopropanol with a high concentration of salt [8]. After washing with 70% ethanol to remove any remaining ethanol or isopropanol, the final target DNA is collected by dissolving it in TE buffer or sterile distilled water [13]. This method is also used for RNA extraction by concomitant use of guanidinium isothiocyanate. This combination can overcome the limitation of RNA extraction using the guanidinium isothiocyanate itself, so RNA could be isolated conveniently using a single-step technique by Chomczynski et al. [12, 14] Total RNA is recovered by precipitation with isopropanol after separation of the upper phase containing the total RNA from the lower phase containing DNA and proteins [12, 14]. Although the phenol-chloroform method is relatively easy compared with the CsCl/EtBr gradient and is very useful for the extraction of nucleic acids, it also is problematic for the clinical microbiology laboratory because phenol has important limitations due to its being toxic, caustic, and flammable [5, 15, 16].

Solid-Phase Extraction

McCormick et al. introduced a new DNA extraction method involving solid-phase extraction in 1989 [17]. They used an insoluble siliceous core particle rather than liquid phenol. The function of this siliceous core particle is similar to that of phenol, but it has several major advantages in that it is safer, and cross contamination can be reduced. It is well known that the precipitation in the phenol/chloroform method causes DNA loss, and Meijer et al. demonstrated that this DNA loss could be reduced by replacing the precipitating step with silica particles [18]. Solid-phase nucleic acid extraction was incorporated into many commercial kits, and it still is the basis of many extraction methods, although siliceous core particles have been replaced by other materials such as silica matrices, glass particles, diatomaceous earth, and anion-exchange carriers (Table 1, Fig. 1) [7]. Solid-phase extraction uses a spin column operated by centrifugal force allowing DNA to be purified rapidly and efficiently without the limitations of liquid extraction, including incomplete phase separation [8]. Solid-phase extraction using silica now is one of most common methods for nucleic acid extraction. Silica that possesses a positive charge combines strongly with DNA, which possesses a negative charge, so it can enable rapid, pure, and quantitative purification [7]. In 1990, Boom et al. [6] used an innovative approach in which diatomaceous earth served as a matrix for solid-phase extraction. The principle of this method is that it immobilizes DNA onto its particles in the presence of a chaotropic agent. The technique can purify rRNA as well as single-stranded and double-stranded DNA. It takes only a short time and can be applied to clinical specimens as well as to DNA and bacteria. The process of solid-phase extraction involves cell lysis, nucleic acid adsorption, washing, and elution

Table 1 Characteristics of solid-phase extraction methods

Material	Molecule of affinity	Advantages	Disadvantages
Silica matrices	DNA, RNA	High-purity DNA, easy to perform, reproducible	Unable to recover small DNA fragments; one-time use
Glass particles	DNA, protein	Simple, sensitive, reproducible	High cost; requirement for equipment
Diatomaceous earth	DNA, RNA	Reduced pipetting error, shorter protocol (less time and fewer steps)	High cost
Magnetic beads	DNA, RNA	No centrifugation, best choice for automation, virtually equipment-free	Interference in PCR amplification
Anion exchange material	DNA, RNA	Reusable resins	Presence of high salt concentrations
Cellulose matrix	DNA, RNA	Easy to use and store	Extraction protocols complex and prone to error

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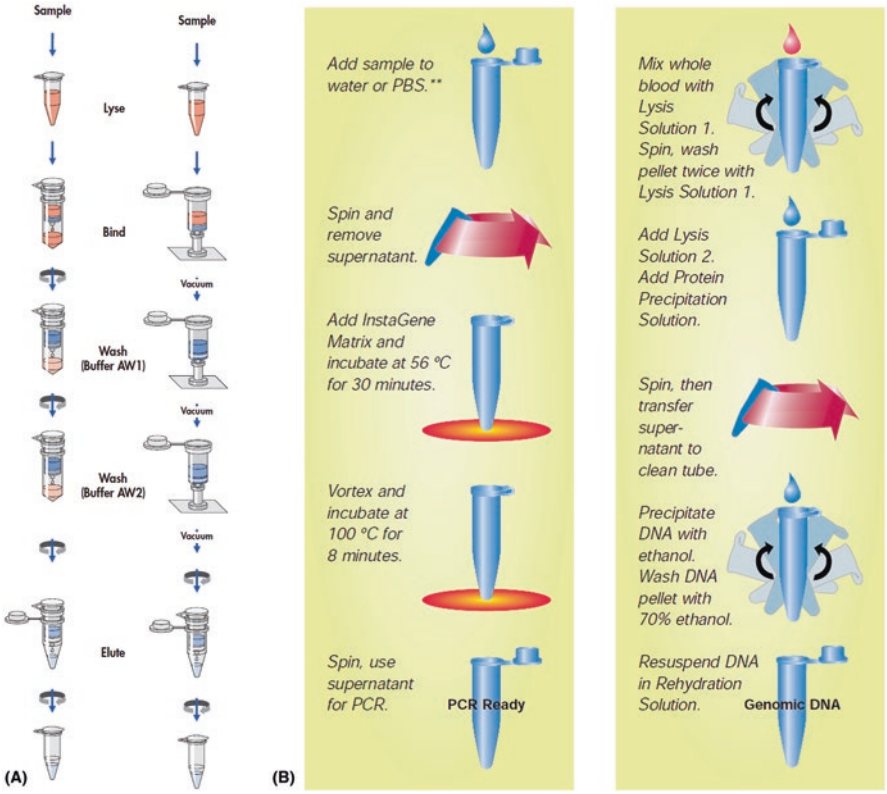

Fig. 1 Schematic diagrams of (**a**) solid-phase, spin-column nucleic acid extraction method (QIAamp DNA mini kit from Qiagen, http://www.qiagen.com) and (**b**) liquid-based nucleic acid extraction method (InstaGene Matrix and Genomic DNA kit from Bio-Rad, http://www.bio-rad.com)

[7, 8]. Column conditioning is obtained using a buffer at a particular pH [20]. The nucleic acid will be released after cell lysis and decanting of lysis buffer into the column. Nucleic acid adsorption is completed in a chaotropic salt solution [20]. Washing buffer contains a competitive agent and can remove contaminants such as proteins and salts. In elution, TE buffer is applied to the column so that purified nucleic acid will be released [20].

Magnetic Bead Method

There is another important modification of solid-phase extraction, that is, the magnetic bead method. The beads have a negative surface charge and bind proteins and cellular debris selectively [7]. So, DNA can be isolated easily from specimens by

removing proteins and cellular debris on the beads. This has the potential advantages of removing the need for repeated centrifugation, vacuum filtration, and column separation for washing and elution as well as organic solvents [7, 8]. The magnetic bead method is very simple and convenient; so many commercially available kits are used for this method [8]. Some manufacturers combined the techniques of solid-phase extraction with the use of silica and magnetic beads, which satisfies the customers' requests for time- and labor-effectiveness and efficiency (Fig. 2). This method is commonly used in automated extraction methods such as miniMag (bioMerieux) and MagNA Pure (Roche). In terms of new technology, additional commercially available kits using this new technique are being introduced into the market. The enzymatic method is an example of these new extraction methods [21]. These new methods help investigators by providing the advantages of convenience with the requirement for only small volumes of specimen while enhancing DNA recovery.

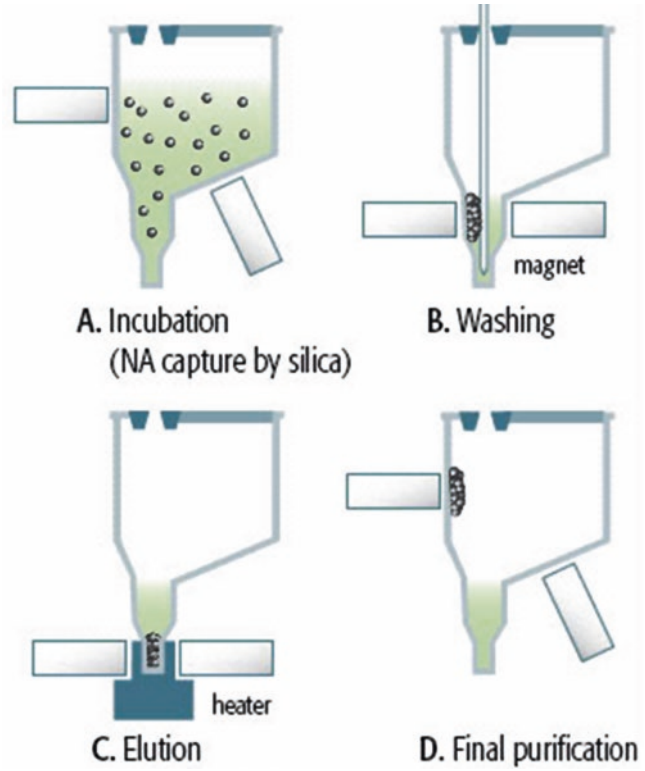

Fig. 2 Extraction principles using magnetic silica particles. (**a**) During incubation of the lysed samples, all the target nucleic acid is captured by magnetic silica particles. (**b**) The NucliSENS easyMAG magnetic device attracts all the magnetic silica, enabling the system to purify the nucleic acids through several washing steps. (**c**) The heating step releases the nucleic acids from the silica. (**d**) In the final step, the magnetic silica particles are separated from the eluate by the magnetic device (NucliSENS easyMAG from bioMerieux, http://www.biomerieux-diagnostics.com)

Applications to Clinical Specimens

Nucleic acid extraction directly from clinical specimens is quite different from that from cultured isolates of bacteria or fungi. The extraction step can greatly influence the subsequent performance of the diagnostic tests; the efficiency of nucleic acid extraction is directly related to the sensitivity of the final test results [22]. Each clinical specimen may have unique and diverse characteristics. Blood and stool are composed of many substances; among these are heme and bile, which act as inhibitors of amplification and should be removed [5]. One can find the comparison results for different nucleic acid extraction methods; however, this alone cannot ensure that one can adapt this result to different specimens and pathogens. In published studies, herpes simplex virus DNA was relatively easily isolated from genital swabs [5, 23, 24], but isolating bacterial DNA from stool samples proved to be complex and more difficult [5, 25].

To overcome these potential limitations, the extraction method must be carefully evaluated prior to routine testing of specific pathogens from specific specimens. For detection of clinically important viruses, extraction efficiency was evaluated in various specimens, including serum, urine, and cerebrospinal fluid, and good performance was confirmed [2, 26, 27]. However, we should not extrapolate these specific results to every type of virus and every type of clinical specimen.

Cellular Component

Tissue is an important clinical specimen for diagnosing localized cytomegalovirus infections in a transplanted organ as biopsy is a common method used both to evaluate potential CMV infection as well as to evaluate potential rejection [28]. However, tissue specimens have problems because the relatively large amount of human tissue contains cellular DNA, proteins, and other materials [29]. So, a more complex step to extract the nucleic acid of the microbial pathogen is needed. Most commercial kits for tissue specimens extract human nucleic acid also. In recent years, it has become possible to extract viral nucleic acid from clinical specimens having cellular components, and there have been trials of these commercially available kits to detect various clinically important viruses [30–32]. There is one report concerning the extraction of six viruses from clinical cellular specimens, and the investigators compared four commercial extraction methods [29]. The viruses included in this study are BK virus, cytomegalovirus, Epstein-Barr virus, human herpesvirus 8, herpes simplex virus, and varicella-zoster virus. All four kits could extract DNA from all six viruses.

Stool is an important clinical specimen for the detection of viruses causing diarrheal illnesses. The results can be affected by the efficiency of nucleic acid extraction from stool, because stool is a mixture of many unrecognized materials, including bacteria, protein, and other cellular materials. So, stool specimens are considered

one of most difficult specimens for nucleic acid extraction in the clinical laboratory. In one report by Peiris et al. [33], the positive rate was 97% for severe acute respiratory syndrome coronavirus in stool samples. However, the detection rates were quite different in another report: only 26.2% and 68.0% between 1 and 2 weeks after disease onset [34]. It is suggested that the difference in positive rates is a consequence of variations in the RNA extraction method [35].

Serum, Plasma, and Whole Blood

Clinicians place great emphasis on the detection of bacteria and fungi in blood. Therefore, nucleic acid extraction from blood has become very important. Many researchers have found that there are numerous PCR inhibitors in blood culture bottles such as sodium polyanetholesulfonate (SPS) and hemins [36]. Millar and collaborators compared several commercial and in-house extraction methods used to detect bacteria and fungi in BacT/Alert blood culture bottles [37]. To reduce the detection time, the serum, plasma, or whole blood is used as a main specimen for detection of bacteria and fungi. Serum or plasma is more efficient and convenient than whole blood because whole blood includes many PCR inhibitors [38]. Most commercial kits showed a high recovery rate of pathogen DNA, but only those methods that used heat lysis with an alkali wash could remove PCR inhibitors. Detection of brucellosis was highly sensitive even though *Brucella* are facultative intracellular pathogens [39]. Similarly, kits containing proteinase K showed better yield of *Brucella* in serum specimens [40]. However, to enhance the sensitivity of PCR amplification, whole blood is considered as a final target because it contains more pathogens than serum or plasma [41]. Although commercially available nucleic acid kits were not developed to extract microbial DNA from whole blood, all commercial kits are able to do so [22]. In recent years, several automated systems have proven to be able to extract bacterial or fungal DNA from whole blood [31, 42, 43], although these instruments are expensive. They are suitable for high-throughput detection [44].

It also is important to consider the concentrations of pathogens. The recovery of *Toxoplasma gondii* was similar for two DNA extraction techniques when using various PCR methods [45]. However, the results were different when there were low concentrations of tachyzoites in blood [46] vs. amniotic fluid [45]. Similar results have been reported for detection of coronavirus from stools [35]. The positive rates were lowered when the RNA concentrations drop and confirm the clinical importance of the extraction methods used for stool samples.

The use of dried blood spots (DBS) is an alternative to whole blood and is an important and common specimen used for the diagnosis of congenital infections [47]. It is different from whole blood in that the tiny specimen may contain very few causative pathogens. At present, many kinds of commercial or non-commercial DBS are in use. Because the amount of blood is small, inadequate DNA extraction can be a problem and result in a low sensitivity. Several investigators attempted to

detect CMV DNA in DBS using several extraction methods and found that the recovery of CMV DNA differed according to the extraction method used [47–51]. The lysis buffer used also can affect the yield of RNA from DBS, and column extraction methods revealed significant loss in RNA recovery [52].

Influence of Specific Pathogen

Whenever clinical specimens are used to extract nucleic acid, it should be recognized that recovery also is influenced by the physical properties of the pathogen [45]. The effect will be greater if the method does not include proteinase K in the lysis step. The *Apicomplexa* phylum including *Toxoplasma* is well known to be resistant to detergent lysis [53].

Fungi are problematic when attempting to extract nucleic acid because it is difficult to break their cell walls in order to release the DNA [15, 43]. Moreover, the detection rates in certain clinical specimens such as whole blood are low because of the very low loads of fungal cells [54–56]. So the extraction process once again is a crucial step and can determine the sensitivity of a particular PCR assay [57, 58]. There was an important report concerning nucleic acid extraction for *Aspergillus* published by the European *Aspergilus* PCR Initiative. About 50% of failures to detect *Aspergillus* were attributable to the low concentration of nucleic acids extracted from the organisms in whole blood [59, 60]. The authors also pointed out that the PCR efficiency of *Aspergillus* is determined not by the PCR amplification but by NA exaction. Most common NA extraction methods for bacteria and viruses are not adequate for fungi. Indeed, NA extraction from fungi needs different procedures, including bead beating, freezing, and larger specimen volumes [60–63]. To recover small amounts of fungal DNA from clinical specimens, a protocol should be established for an optimal extraction method. The QIAamp DNA blood kit was successful in extracting *Candida* DNA and was suitable to use with a *Taq*Man-based PCR assay, whereas all other kits tested failed to detect low amounts of *Candida* DNA [15]. Perry et al. [60] reported that the Qiagen EZ1 Advanced XL and Roche MagNA Pure LC platforms were suitable for *A. fumigatus* DNA extraction from EDTA-treated whole blood. However, there were significant differences between automated NA extraction instruments. Those investigators finally concluded that full validation of the NA extraction efficiency should be done even though others have reported good validation results with other organisms. In another study, the investigators were successful with all of the extraction methods used in their study, even though those kits were not specifically designed for the extraction of fungal DNA from whole blood [64]. A similar difficulty in extracting nucleic acid from *Mycobacteria* has been observed by the author of this chapter. Many researchers have tried to find optimal extraction methods for most clinically important specimens; the most appropriate method for each laboratory's situation should be applied [65].

PCR Inhibitors

There are many factors that affect the efficiency of nucleic acid extraction; the most important factor is the presence of many different types of inhibitors. It is well known that bile salts, hemoglobin, and polysaccharides can inhibit PCR [66, 67]. There also may be contaminating bacterial or fungal DNA in the reagents [40, 64, 68]. Most PCR assays can be influenced by reaction inhibitors and other contaminants, especially when the various clinical specimens containing these inhibitors are used as samples, and nucleic acid extraction thus becomes the crucial step that determines their influence [69]. In previous reports [30, 70], the presence of inhibitors was confirmed when the MagNA Pure Compact system was used for a principal nucleic acid extraction. In one other report [46], the authors compared the MagNA Pure Compact system and QIAamp DNA mini kit for the detection of *Toxoplasma* DNA from blood. The sensitivity of PCR using the MagNA Pure Compact system was lower than that of the QIAamp DNA mini kit, so the presence of inhibitors may have been responsible for the difference of sensitivity between the two methods. Moreover, the combination of the extraction kit and the master mix can make a difference in PCR performance in terms of inhibition [45]. One should also consider the fact that many human DNAs are mixed with relatively rare pathogen DNAs in clinical specimens, meaning abundant human DNA will be obtained during extraction for pathogen detection [71].

Measurement of DNA Quality

The classical method to check DNA purity is to measure the adsorption of UV light at 260 nm and 280 nm. The DNA content is proportional to the adsorption of UV light at 260, and adsorption at 280 nm reflects protein contamination. So, one can easily calculate the DNA purity using the OD260/OD280 ratio. In recent years, newly developed methods such as PicoGreen have been introduced and are becoming more popular in clinical laboratories, although the spectrophotometric method does have many advantages [72]. PicoGreen is based on the use of fluorescence and needs only a minute volume of sample.

Comparison of Nucleic Acid Extraction Methods

The method used for nucleic acid extraction differs greatly in clinical microbiology laboratories. When cultured bacteria are the source of genomic DNA, it is common to use simple heating, but this method has many limitations and is not appropriate for use directly on clinical specimens. There are many reports comparing different types of extraction methods, including those using commercially available kits, and

different types of clinical specimens for NA extraction from bacteria, virus, and fungi [22, 26, 27, 30, 31, 40, 43, 45, 73–77]. The methods can be divided into solution or column based according to differences of their principles, and most commercial extraction kits we use can be divided the same way. DNA recovery was better when a spin-column method was used for extraction of *C. pneumoniae* DNA from vascular tissue [78]. However, DNA recovery ability can differ among kits even though all use the spin-column method as the principal tool [79]. So the method itself does not give assurance, and one should keep in mind that the DNA recovery can be different among various kits that use the similar principles.

Regardless of specific kits, specific companies, and their protocols, they have common steps in their procedures for optimal extraction [8]. Cell lysis must be the first step. After nucleoprotein complexes have been denatured, nucleases are inactivated. The contaminants are removed, and nucleic acid is purified. Even though these basic steps are not changed, there has been a vast alteration in nucleic acid extraction, namely, development of automated instrumentation. The method for the nucleic acid extraction can be divided into manual or automated, and this is an important point in the classification of nucleic acid extraction methods.

Manual Method

Many commercial kits have been developed for nucleic acid extraction. These kits are composed of a few reagents and are designed primarily for manual extraction. These kits are suitable for use in clinical laboratories and have replaced older in-house methods (Table 2). There are many publications that have evaluated the performance of these commercial nucleic acid extraction methods and comparing them with conventional methods such as phenol-chloroform and the alkali wash/heat lysis [15, 37, 40, 64]. These manual commercial extraction kits show good performance for nucleic acid extraction compared with in-house methods.

Their ability to recover pure DNA and to remove the contaminants, including proteins, is of great importance, but there are also important differences in cost, time demands, labor intensity, and the principles of each method. Given these differences, there are numerous choices available; the most appropriate method for a particular laboratory should be selected. Both liquid- and column-based methods are commonly used at present.

Most of these kits use noncorrosive agents, so they are safe and easy to deal with. However, there still are some pitfalls. Although the entire extraction procedure is standardized by the manufacturer's manual, the process is still complicated and is performed manually. Therefore, problems with reproducibility by different persons can be seen. To minimize such reproducibility problems, it is necessary to provide continuous training and quality control [5]. Ethanol is used for precipitation of the nucleic acid in some manual kits, and inhibition of the PCR reaction can occur when this ethanol is not completely removed [80]. The manual extraction method has been designated as a high-complexity test according to Clinical Laboratory

Table 2 Manual methods of nucleic acid extraction and purifications for rapid real-time PCR assays

Kit/ manufacturer/ homepage	Technologic principle	Specimen throughput	Specimen type
High Pure Roche Applied Science www.roche-applied-science.com	Nucleic acid capture by glass fiber fleece immobilized in a special plastic filter tube and subjected to centrifugation	24 samples in 1 h	Serum, whole blood, plasma, urine, stool, sterile body fluids, respiratory tract specimens, swabs (genital, dermal)
QIAamp Qiagen www.qiagen.com	Nucleic acid capture by silica gel membrane placed in tube column and subjected to centrifugation or vacuum conditions	24 samples in 1 h for DNA; 24 samples in 1.5 h for RNA	Respiratory tract specimens, plasma, stool, serum, whole blood, urine, sterile body fluids, swabs (nasal, fecal)
IsoQuick Orca Research www.bioexpress.com	Nucleic acid is partitioned into an aqueous phase and then precipitated with ethanol and resuspended in water or buffer	24 samples in 1 h for DNA; 24 samples in 2 h for RNA	Plasma, whole blood, stool, respiratory tract specimens, sterile body fluids, swabs (dermal, fecal, genital)
IsoCode Stix Schleicher and Schuell www.whatman.com	DNA bound to matrix and released by simple water and heat elution	Processed individually	Whole blood

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Improvement Amendments of 1988 (CLIA ‘88) regulations (http://www.cms.hhs.gov/clia/), so only trained laboratory personnel can perform it. Moreover, the number of specimens for molecular testing is increasing, which places more stress on the technologist who are processing these specimens. This can affect the accuracy of tests as a result of a processing mistake or by contamination attributable to the complex processing procedure.

Automated Methods

The introduction of commercial manual extraction kits brought was a valuable adjunct for molecular testing in the clinical microbiology laboratory. However, the manual extraction method is still labor-intensive and time-consuming and requires a well-trained technologist. There were also reports of outbreaks of cross contamination when multiple samples were processed at the same time [40, 81]. In recent years, many manufacturers developed and launched various automated extraction instruments; these instruments vary in principle, procedure time, cost, and size (Table 3). The automated extraction instruments are easily divided by their workload capacity; the most appropriate instrument thus can be selected according to

Table 3 Automated nucleic acid extraction methods and their characteristics

Manufacturer	Instrument	Technologic principle	Specimens/run (batch size)
BioMérieux	eMAG	Magnetic bead extraction	48 (1–48)
BioMérieux	NucliSENS easyMAG	Magnetic bead extraction	24
BioMérieux	NucliSENS miniMAG	Magnetic bead extraction	12
Roche	MagNA Pure 96	Magnetic bead extraction	96 (1–96)
Roche	MagNA Pure 24	Magnetic bead extraction	24 (1–24)
Roche	MagNA Pure LC	Magnetic bead extraction	32 (1–32)
Roche	MagNA Pure Compact	Magnetic bead extraction	8 (1–8)
Qiagen	BioRobot Universal/BioRobot MDx	Vacuum-based and/or magnetic bead extraction	96 (8)
Qiagen	QIAsymphony SP	Magnetic bead extraction	96 (1–24)
Qiagen	QIAcube	Vacuum-based extraction	1–12
Bioneer	ExiPrep 16 Plus	Magnetic bead extraction	16 (1–16)
Abbott Molecular	M2000sp	Magnetic bead extraction	96 (24)
Eppendorf	epMotion 5070/epMotion 5075	Vacuum-based and/or magnetic bead extraction	384 (1–384)
Fisher Scientific	Thermo KingFisher Flex	Magnetic bead extraction	1–96 (1–96)
Beckman Coulter	Biomek NXp Span 8/Biomek NXp Multichannel 96	Magnetic bead extraction	96 (8)

each laboratory's workload situation from low-throughput instruments such as MagNA Pure Compact, NucliSENS miniMAG, and BioRobot EZ1 systems to medium- to high-throughput instruments such as the MagNA Pure 96 and BioRobot M48/96 [82].

The automated extraction methods have many advantages compared with manual methods, and these instruments have proven to be very useful adjuncts for PCR testing. The steps proceed automatically with fast turnaround. This reduces the working time and avoids mistakes such as pipetting error. Multiple specimens can be analyzed at the same time [44]. It provides constant reproducibility for recovery of nucleic acid, avoiding person to person variations seen with manual extraction methods. It can also diminish cross contamination by reducing unnecessary handling steps and avoiding mistakes by personnel [5]. It has an additional advantage for quality control monitoring, whereas the manual method is labor-intensive in terms of quality control monitoring [83].

Since many automated extraction instruments and kits have been developed, numerous evaluations have been reported [73, 84–87]. These studies included various kinds of extraction kits, clinical specimens, and pathogens. Even though some reports showed high detection rates with manual extraction method, the results of automated extraction methods were similar to or better than those of manual methods in most of these studies [29, 30, 46, 70, 73, 84–87].

For example, Cook et al. [29] evaluated the performance of four commercial automated extraction kits for the detection of viruses using clinical specimens. They compared viral yield using cultured cells containing CMV, EBV, HSV, BK, VZV, or HHV-8. The procedures of the kits were similar, and DNA extraction was successful with all kits. There were some variations of viral yields, which were only 50% compared with those of the manual kits. The differences of yields are not of great significance if the biologic range of viral loads in clinical practice is considered as per the manufacturer's recommendation. In the study of multiplex molecular detection of infection in septic patients using automated extraction, the recovery of DNA was similar to that of the conventional manual method at the point of maximal binding surface of MagNA pure nanoparticles [82]. Many leukocytes are present in the blood sample, so the final DNA amounts recovered by the manual method can be about three times those obtained by MagNA pure extraction [88].

Very different results are seen with the evaluation of automated extraction instruments. The efficiency of the NucliSENS easyMAG was low for CMV [50] and respiratory viruses [30]; however, in another study, the NucliSENS miniMAG showed the best results for the isolation of polyomavirus BK virus and the human beta-actin gene from urine specimens [2] and severe respiratory syndrome coronavirus RNA in stool samples [35]. There are other reports dealing with identification of more than 14 respiratory viruses using different NA extraction instruments [89]. Those investigators compared three automated extraction systems with same RT-PCR reagent using nasopharyngeal aspirates. The three systems showed different sensitivity and specificity performances for each virus, although the QIAcube system showed the fewest false-negative results and the best concordance rate. Thus, the QIAcube may be best suited for detecting different types of viruses, including both RNA and DNA viruses.

The most important drawback that must be considered is the economic aspect of the automated methods. To use such a system, an expensive instrument and extraction reagents, including disposables, are needed. Sometimes, this increased cost for the automated system precludes its use. However, the influenza A (H1N1) pandemic in 2009 demonstrated the value of an automated extraction system. At that time, requests for influenza A (H1N1) identification was increasing rapidly, and many laboratories could not perform all the requested tests because of limited personnel. This made clear the usefulness of the automated extraction system. Although the detection rates and yield recovery are the most important factors in selecting commercial extraction methods, other factors, including ease of use and the cost per extraction, also must be considered [29].

Microfluidics and Full Automations Instruments

In recent years, numbers of fully automated instruments combining NA extraction and amplification have become commercially available. These instruments allow the automation of the total process from NA extraction to the report of the final

results [19]. Some of these devices simply combined the separate NA extraction and amplification into one instrument. For example, the BD MAX instrument is composed of two sections: the NA extraction is based on the magnetic bead method, while the amplification step is performed in a microfluidic cartridge. Other devices include microfluidic chips containing all necessary reagents for molecular testing; these instruments use membranes or beads of solid-phase extraction for NA isolation [19]. It is possible to make a POCT cartridge having a closed container such as GeneXpert, FilmArray, and Verigene. For these instruments that have combined fully automated systems, the NA extraction step cannot be separated from the amplification step, so we cannot evaluate the reliability of the extraction step alone but can only estimate the NA extraction by evaluating of the final results.

Conclusion

Many advanced molecular methods and automated instruments have been adapted in clinical microbiology laboratories because of their high sensitivity and specificity; these methods/instruments occupy an important position in the current diagnosis of infectious diseases [90, 91]. Efficient nucleic acid extraction is essential to obtain good results using any molecular method/instrument. The optimal extraction method should fulfill the following conditions: speed, short working time, cost-effectiveness, high sensitivity and specificity, good reproducibility, and safety [1]. The extraction method ideally should be effective with all kinds of specimens and pathogens. However, at present, there is no one extraction method that satisfies all these conditions. On the contrary, there are significant differences between extraction kits because nucleic acids can be different in specific clinical specimens. So, it is important to carefully evaluate the performance of any extraction method used in the clinical microbiology laboratory. Moreover, each extraction method has its own specific characteristics, so the most suitable method should be selected for each test.

In recent years, several fully automated instruments for molecular testing have been introduced. Even though one cannot individually estimate their capabilities for nucleic acid extraction, amplification, and detection, one can and should judge the quality of their extraction process by evaluating their overall ability to detect pathogens.

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