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Evaluation of Automated and Manual Commercial DNA Extraction Methods for Recovery of *Brucella* DNA from Suspensions and Spiked Swabs▽

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This study evaluated automated and manual commercial DNA extraction methods for their ability to recover DNA from *Brucella* species in phosphate-buffered saline (PBS) suspension and from spiked swab specimens. Six extraction methods, representing several of the methodologies which are commercially available for DNA extraction, as well as representing various throughput capacities, were evaluated: the MagNA Pure Compact and the MagNA Pure LC instruments, the IT 1-2-3 DNA sample purification kit, the MasterPure Complete DNA and RNA purification kit, the QIAamp DNA blood mini kit, and the UltraClean microbial DNA isolation kit. These six extraction methods were performed upon three pathogenic *Brucella* species: *B. abortus*, *B. melitensis*, and *B. suis*. Viability testing of the DNA extracts indicated that all six extraction methods were efficient at inactivating virulent *Brucella* spp. Real-time PCR analysis using *Brucella* genus- and species-specific TaqMan assays revealed that use of the MasterPure kit resulted in superior levels of detection from bacterial suspensions, while the MasterPure kit and MagNA Pure Compact performed equally well for extraction of spiked swab samples. This study demonstrated that DNA extraction methodologies differ in their ability to recover *Brucella* DNA from PBS bacterial suspensions and from swab specimens and, thus, that the extraction method used for a given type of sample matrix can influence the sensitivity of real-time PCR assays for *Brucella*.

Members of the *Brucella* genus are gram-negative, aerobic, nonmotile coccobacilli that can infect a broad range of animal hosts. The genome of *Brucella* consists of two circular chromosomes, with approximate sizes of 2.1 and 1.2 Mbp (21). Genomic studies have shown such a high degree of genetic similarity among the *Brucella* spp. (10, 12, 25) that a monospecies designation for the genus has been proposed (33). Because of this conservation of sequence, individual species of *Brucella* are difficult to differentiate using older molecular techniques, but recent advances, such as multilocus analysis of variable number tandem repeats, have been successfully used to distinguish isolates (2, 9, 17). There are now six recognized *Brucella* species, which are classically distinguished by their host specificity (9, 21). Three of these species, *B. abortus*, *B. suis*, and *B. melitensis*, are major human pathogens, with *B. melitensis* being the most prevalent (1, 23).

B. abortus, *B. melitensis*, and *B. suis* are veterinary pathogens which cause spontaneous abortion in livestock (24) and are also the etiological agents of human brucellosis, which has been described as the most common zoonosis worldwide. Transmission of the disease to humans usually occurs through direct contact with infected animals, consumption of contaminated food, or inhalation of aerosolized particles (23), whereas person-to-person transmission rarely occurs (24).

Brucellosis is a severe febrile disease that is rarely fatal, but the ease with which *Brucella* can be spread as an aerosol makes it an attractive biological weapon. In the 1950s, *B. suis* became the first biological agent weaponized by the United States (4). Due to their moderate ease of dissemination and low mortality rate, *B. abortus*, *B. melitensis*, and *B. suis* are classified as category B critical biological agents by the Centers for Disease Control and Prevention (CDC) (30).

Diagnostic methods for brucellosis rely on serological testing or the isolation and cultivation of the organism from clinical specimens, but these methods can be relatively time-consuming and lack sensitivity and specificity (1). The infectious dose for *Brucella* in humans is 10 to 100 organisms; consequently, diagnostic laboratory personnel who cultivate these organisms are at significant risk of accidental exposure, and brucellosis is one of the most commonly reported laboratory-acquired infections (11). To minimize the risks associated with handling potentially infectious specimens, molecular diagnostic assays, such as real-time PCR, have been developed for the rapid detection of *Brucella* spp. in a variety of specimen types (8, 9, 14, 22, 26).

The increasing use of molecular diagnostics has resulted in increased numbers of specimens submitted to clinical laboratories and has necessitated automation of the processing procedures (32). Given that DNA extraction methods can influence the sensitivity of real-time PCR assays (6), selection of an

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TABLE 1. Summary of the automated and manual DNA extraction methods used in this study

Extraction method	Vendor	Catalog no.	Principle
MagNA Pure Compact	Roche	03731146001	Lysis buffer and proteinase K treatments, followed by binding of nucleic acid to magnetic glass particles, magnetic separation of nucleic acid, and elution at high temperatures
MagNA Pure LC	Roche	12236931001	Same as for the MagNA Pure Compact instrument
IT 1-2-3 DNA sample purification kit	Idaho Technology	3800	Lysis buffer and bead beating, followed by binding of DNA to filter membrane, and contaminants pass through spin column
MasterPure complete DNA and RNA purification kit	Epicentre	MC85200	Lysis buffer and proteinase K treatments, followed by RNase A treatment, protein precipitation, and DNA precipitation
QIAamp DNA blood mini kit	Qiagen	51106	Proteinase K treatment, followed by binding of DNA to silica gel membrane, and contaminants pass through spin column
UltraClean microbial DNA isolation kit	MoBio	12224-250	Lysis buffer and bead beating, followed by binding of DNA to silica gel membrane, and contaminants pass through spin column

optimal extraction method is critical for the laboratory detection of *Brucella* spp. Relatively few studies have evaluated commercial DNA extraction methods specifically for the recovery of *Brucella* DNA. One such study, by Queipo-Ortuño et al. (27), compared commercial extraction kits for the recovery of *Brucella* DNA from spiked serum samples and reported that the UltraClean DNA Blood Spin kit provided optimal results. However, their study evaluated only manual extraction kits, which do not provide the high-throughput extraction capacity that is needed in clinical laboratories. Furthermore, it has been demonstrated that laboratories are likely to receive large numbers of specimens during bioterrorism investigations (13, 15, 18), which suggests the need for an evaluation of automated DNA extraction methods.

The purpose of this study was to compare the performances of commercial extraction methods with regard to DNA yield and purity as judged by using *Brucella* genus- and species-specific real-time PCR assays (14). Six extraction methods were evaluated, representing several of the most popular commercially available methodologies for DNA extraction, including magnetic bead purification, filter membrane purification, and alcohol precipitation. The performance evaluation criteria included residual-viability testing of the DNA extracts, limit of detection studies for three *Brucella* spp. in phosphate-buffered saline (PBS) suspensions of bacterial cells and dried spiked-swab samples, and comparisons of DNA yields, DNA purity, processing costs and times, and required materials.

MATERIALS AND METHODS

Biosafety procedures. All procedures using virulent *Brucella* spp. were performed in a biosafety level 3 laboratory. Culturing of *Brucella* spp. and DNA extraction procedures, with the exception of automated processing, were conducted in a class II type A2 biological cabinet (NuAire, Plymouth, MN). Additional biosafety level 3 precautions included the use of a powered air-purifying respirator and protective laboratory clothing.

***Brucella* strains and culture.** The *Brucella* strains used in this study originated from stock preparations maintained in the Bioterrorism Rapid Response and Advanced Technology Laboratory, Division of Bioterrorism Preparedness and Response, CDC. Three pathogenic *Brucella* spp. were used for performance evaluations: *B. abortus* strain D9606470, *B. melitensis* strain 8902041, and *B. suis* strain 051305NY. Nonpathogenic *Brucella ovis* strain KC354 was used as a positive control for real-time PCR assays.

Cultures were initiated from frozen stocks and streaked for isolation onto Trypticase soy agar plates with 5% (vol/vol) sheep blood (TSAB) (BD Diagnostic Systems, Sparks, MD), and the plates were incubated for 72 h at 37°C. For each strain, a single colony was transferred to 1 ml of sterile physiological saline (0.85% sodium chloride) by using a sterile inoculating loop and mixed by vortexing at low speed for 30 s. A 200-μl aliquot of each suspension was spread onto TSAB plates in triplicate, and the plates were incubated for 72 h to 96 h at 37°C. Cultures were harvested into 15 ml of sterile PBS (0.01 M; pH 7.4), using sterile Dacron fiber-tipped swabs (Fisher Scientific, Pittsburgh, PA) which were premoistened with PBS. Standard bacterial plate counting methods were used, and once quantified, the *Brucella* suspensions in PBS were stored at −70°C until use.

Spiking of swabs. Swabs were included as a specimen type because they are commonly tested in diagnostic laboratories and are often used to collect environmental samples during suspected bioterrorism investigations (29). Three swab materials were selected to assess their relative efficiencies for the recovery and subsequent detection of *Brucella* DNA by real-time PCR: polyester, polyurethane foam, and rayon (Fisher Scientific, Pittsburgh, PA). Tenfold serial dilutions of *B. melitensis* at a starting concentration of 10^7 CFU/ml were performed in PBS, and 10-μl aliquots were used to inoculate swabs in triplicate. The swabs were allowed to air dry at room temperature for 30 min, placed into 1 ml PBS in 15-ml polypropylene tubes (Fisher Scientific, Pittsburgh, PA), and vortexed at high speed for 2 min. After expressing residual liquid from the swabs, the samples were transferred to 1.7-ml microcentrifuge tubes (Marsh Bio Products, Rochester, NY) and used for subsequent DNA extraction.

DNA extraction. Six commercial DNA extraction methods, including two automated systems and four manual kits, were evaluated in this study. The six methods used four different principles for the removal of protein and PCR inhibitors from the samples and for the recovery of DNA. The processing time for a 21-sample run was determined for each extraction method, beginning with the addition of the first reagent and ending with the recovery of PCR-ready DNA. Following extraction procedures, samples were stored at −20°C in the elution or resuspension buffers provided with the kits, as recommended by the manufacturers.

Automated DNA extractions were performed using the MagNA Pure Compact and the MagNA Pure LC instruments (Roche Applied Science, Indianapolis, IN). Both instruments utilize magnetic bead technology as described in Table 1. The MagNA Pure Compact is a low-throughput, stand-alone instrument that processes up to eight samples per run, whereas the MagNA Pure LC is a medium- to high-throughput system which includes an accompanying computer and processes up to 32 samples per run. For both procedures, an external lysis protocol (optional) was performed prior to DNA extraction by combining 200-μl aliquots of either quantified *Brucella* suspensions or samples recovered from swab specimens, with 300 μl of MagNA Pure LC DNA isolation kit I lysis/binding buffer (catalog number 03 246 752 001; Roche Applied Science, Indianapolis, IN) in 2-ml screw-cap tubes. The samples were mixed thoroughly by pipetting and incubated at room temperature for 30 min. Following the lysis procedure, the samples underwent automated DNA extraction on the instruments, using MagNA Pure Compact nucleic acid isolation kit I for the MagNA Pure Compact and MagNA Pure LC DNA isolation kit I for the MagNA Pure LC.

The four manual DNA extraction kits used three different principles for DNA

extraction (Table 1). Both the IT 1-2-3 DNA sample purification kit (Idaho Technology, Inc., Salt Lake City, UT) and the UltraClean microbial DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) combine bead-beating and spin column technologies. The MasterPure complete DNA and RNA purification kit (Epicentre, Madison, WI) uses a precipitation methodology, and the QIAamp DNA blood mini kit (Qiagen, Inc., Valencia, CA) utilizes silica spin filter technology. All of the manual DNA extraction methods were performed according to the manufacturers' instructions, and these procedures have been described in detail previously (6, 7).

To evaluate the ability of the six extraction methods to isolate *Brucella* DNA, triplicate suspensions of *B. abortus*, *B. melitensis*, and *B. suis*, at concentrations ranging from 10^6 to 10^0 CFU/ml, were processed by each method. To compare the six extraction methods for their ability to recover *Brucella* DNA from swab specimens, triplicate samples recovered from swabs spiked with dilutions of *B. melitensis* were extracted.

Viability testing. Viability testing was performed to assess the ability of each extraction method to lyse or kill virulent *Brucella* spp. A total of 108 samples were tested, which included DNA extracts prepared with the six DNA extraction methods from triplicate suspensions of three *Brucella* spp. at concentrations of 10^6 and 10^5 CFU/ml. Following the extraction procedures, 10% of the volume of each sample extract was spread onto TSAB plates, and the plates were incubated for up to 5 days at 37°C. As a control for viability testing, an equal volume of each stock bacterial suspension was spread onto TSAB plates, and the plates were incubated as described above. Viability was determined by direct observation of the plates for colonies. The lysis or killing limit for each extraction method was determined to be the greatest concentration at which three out of three replicate sample extracts resulted in no growth in culture. For safety purposes, the remaining volume of the viability testing extracts, as well as all other DNA extracts prepared in this study, were filtered using 0.1-μm centrifugal filter units (Millipore Corporation, Billerica, MA) as described previously (5).

DNA yield and purity. DNA extracted from *B. abortus*, *B. melitensis*, and *B. suis* cells, at viable cell concentrations ranging from 10^6 to 10^0 CFU/ml, was quantified using a NanoDrop 8000 spectrophotometer (ND Technologies, Wilmington, DE). DNA absorbencies were measured in the elution buffers provided with each kit, and the spectrophotometer was blanked with the corresponding buffer before measurement. For the MagNA Pure Compact, the spectrophotometer was blanked with the elution buffer provided with the MagNA Pure LC extraction kit. The absorbance at 260 nm (A_{260}) was measured for each sample and used to calculate the average concentration of DNA for each set of triplicate samples by multiplying the A_{260} measurement by the conversion factor (50 μg/ml/1 A_{260} unit for double-stranded DNA). To estimate the purity of DNA extracts, the absorbance at 280 nm (A_{280}) was measured and the average ratio between the A_{260} and A_{280} (A_{260}/A_{280}) was calculated for triplicate samples. Samples with A_{260}/A_{280} ratios between 1.8 and 2.0 were presumed to be free of significant contamination (19).

Preparation of positive controls for real-time PCR assays. Cultures of *B. abortus*, *B. melitensis*, *B. suis*, *and B. ovis* were prepared for use as sources of DNA for positive controls in real-time PCR assays. Briefly, cultures were harvested into 250 μl of sterile deionized water in microcentrifuge tubes. The samples were vortexed briefly, boiled for 5 min, and pelleted by centrifugation for 30 s at 10,000 × *g*. The supernatants were transferred to 0.1-μm filter units and filtered as described above. Filtered cell lysates were diluted in Tris-EDTA buffer to dilutions which produced real-time PCR cross threshold (cycle threshold [C_T]) values between 25 and 30. These positive-control samples were stored at −20°C throughout the study.

Real-time PCR analysis. The real-time PCR assays described by Hinić et al. (14) were used to evaluate the six extraction methods for the recovery of *Brucella* DNA from bacterial suspensions and spiked swab samples. The assays were developed for the rapid detection of members of the *Brucella* genus and for the identification of individual species, including *B. abortus*, *B. melitensis*, and *B. suis*. For members of the *Brucella* genus, the assay targets the multiple insertion element IS*711* located on the *Brucella* chromosome, while unique genetic markers are targeted for the specific detection of *B. abortus*, *B. melitensis*, and *B. suis* (14). PCRs were performed using 25-μl volumes, each of which contained 1× LightCycler FastStart DNA Master HybProbes PCR master mix (Roche Molecular Biochemicals, Indianapolis, IN), 300 nM of each genus- or species-specific PCR primer, 200 nM of each specific 6-carboxyfluorescein-labeled TaqMan probe, 5 mM $MgCl_2$, and 5 μl of either each sample extract, positive control DNA, or water (in the case of the no-template controls). *B. ovis* DNA was used as the positive control for the genus-specific real-time PCR assay, while DNA from *B. abortus*, *B. melitensis*, and *B. suis* was used for the corresponding species-specific assays. An exogenous internal-positive-control (IPC) real-time PCR assay (Applied Biosystems, Foster City, CA) was used to assess the ability of each DNA extraction method to remove PCR inhibitors. The IPC reagents, which included a control DNA, PCR primers, and VIC-labeled TaqMan probe, were added to each PCR and were run in the presence of each DNA extract according to the manufacturer's instructions. Real-time PCR was performed on the 7500 Fast real-time PCR system (Applied Biosystems, Foster City, CA) using the standard 7500 operational setting and a thermocycling profile consisting of a hot-start Taq activation step of 95°C for 10 min followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. Data collection and analysis were performed using the 7500 Fast System Sequence Detection Software, version 1.4, including the 21 CFR Part 11 electronic records module for FDA compliance.

To compare the DNA extraction methods for the isolation of DNA from *Brucella* spp., real-time PCR was performed using triplicate DNA extracts prepared from *B. abortus*, *B. melitensis*, and *B. suis* at concentrations ranging from 10^6 to 10^0 CFU/ml. The limit of detection was determined to be the lowest concentration for which three out of three replicates produced a positive result for the genus-reactive real-time PCR target, as measured by a C_T value of ≤ 40. To compare the DNA extraction methods for the recovery of *Brucella* DNA from swab specimens, real-time PCR was performed on triplicate DNA extracts prepared from swabs spiked with dilutions of *B. melitensis*. The limit of detection for spiked swab samples was determined as described above.

Statistical analysis. To determine whether the variability of C_T values for *Brucella* DNA extracted from PBS suspensions and spiked swab specimens using the six DNA extraction methods was significant, the C_T values were compared using one-way analysis of variance (ANOVA). When significant differences were identified, Tukey's multiple comparison test was used to perform nonparametric pairwise analyses of the C_T values.

RESULTS

Inactivation efficiency of extraction methods for virulent *Brucella* spp. All of the DNA extraction methods were efficient at killing virulent *Brucella* spp. at concentrations of ≤10^6 CFU/ml, as there was no growth observed in cultures of DNA extracts prepared using any of the six extraction methods. The viability testing controls were positive for each *Brucella* sp. Since 10% of the volume of each sample extract was used for viability testing, this would indicate at least a 5-log-unit reduction in bacterial viability for the six DNA extraction methods evaluated in this study.

Comparison of extraction methods by real-time PCR. Table 2 shows the real-time PCR limit of detection using DNA extracted from three *Brucella* spp. at concentrations ranging from 10^6 to 10^0 CFU/ml with the six extraction methods. Overall, the MasterPure kit yielded DNA with the best level of detection for *Brucella* spp. MagNA Pure Compact yielded DNA detected at the second-best level of detection, followed by the UltraClean kit, then the IT 1-2-3 and QIAamp kits, which yielded DNA with equivalent detection levels. MagNA Pure LC resulted in DNA with the poorest level of detection by real-time PCR. The differences in mean C_T values for the six DNA extraction methods were found to be significant by one-way ANOVA ($P < 0.05$; $n = 27$). Pairwise comparisons of C_T values indicated significant differences between the MasterPure kit and the five other extraction methods ($P < 0.05$; $n = 27$). There was no evidence of PCR inhibition for any of the extraction methods, as measured by the IPC assay (data not shown).

Comparison of DNA yield and purity. Table 3 shows the average DNA concentrations and A_{260}/A_{280} ratios for triplicate sample extracts from *Brucella* spp. at a concentration of 10^6 CFU/ml. On the whole, the MasterPure kit yielded DNA with the highest concentrations for all *Brucella* spp. The UltraClean kit and MagNA Pure Compact yielded DNA at the second-highest concentrations. MagNA Pure LC and the IT 1-2-3 and

TABLE 2. Real-time PCR limit of detection for DNA recovered from *Brucella* spp. using automated and manual DNA extraction methods

Extraction method[b]	Value for[a]:								
	B. abortus			*B. melitensis*			*B. suis*		
	LOD	PCR1 C_T	PCR3 C_T	LOD	PCR1 C_T	PCR2 C_T	LOD	PCR1 C_T	PCR4 C_T
MagNA Pure Compact	10^1	34.1 ± 0.10	37.9 ± 1.33	10^2	34.4 ± 0.62	36.9 ± 0.55	10^2	27.9 ± 0.68	37.5 ± 0.69
MagNA Pure LC	10^4	31.1 ± 0.92	34.2 ± 0.66	10^3	31.2 ± 0.03	34.1 ± 0.58	10^4	28.8 ± 0.75	39.0 ± 1.07
IT 1-2-3	10^3	31.9 ± 1.34	35.0 ± 0.97	10^3	32.2 ± 0.81	33.9 ± 0.96	10^3	31.0 ± 0.57	40.3 ± 0.15
MasterPure	$\leq 10^0$	34.4 ± 0.15	39.5 ± 1.89	10^1	33.4 ± 0.21	36.8 ± 0.49	10^1	31.2 ± 0.25	39.7 ± 0.93
QIAamp	10^3	35.9 ± 2.36	37.6 ± 0.88	10^3	36.7 ± 0.55	38.8 ± 1.67	10^3	31.4 ± 0.23	40.2 ± 0.71
UltraClean	10^3	31.2 ± 0.72	35.8 ± 0.35	10^2	32.2 ± 1.08	34.4 ± 1.26	10^2	29.8 ± 0.26	40.1 ± 0.24

[a] The limit of detection (LOD) was the lowest concentration (CFU/ml) for which three out of three replicates produced a positive result. Average C_T values (mean ± standard deviation) are shown for the *Brucella* genus-reactive (PCR1) and species-specific targets (PCR2, PCR3, and PCR4) at the lowest concentration that produced a positive result by the use of the real-time PCR assay described by Hinić et al. (14).

[b] Extraction methods were performed in triplicate using *Brucella* spp. at concentrations ranging from 10^6 to 10^0 CFU/ml.

QIAamp kits ranked equally third, yielding comparable DNA concentrations. The MasterPure kit, the UltraClean kit, and the MagNA Pure Compact yielded DNA with the highest purity, with A_{260}/A_{280} ratios ranging from 1.50 to 1.72, 1.65 to 1.75, and 1.59 to 1.88, respectively. The IT 1-2-3 and QIAamp kits yielded the least-pure DNA samples, with ratios ranging from 1.26 to 1.51 and 1.49 to 1.56, respectively.

Real-time PCR analysis of DNA extracted from spiked swab specimens. The automated method with the best level of detection by real-time PCR was compared with the manual extraction methods for the recovery of *Brucella* DNA from swab specimens. Table 4 shows the limit of detection of real-time PCR using DNA extracted from swabs spiked with dilutions of *B. melitensis* with MagNA Pure Compact and the IT 1-2-3, MasterPure, QIAamp, and UltraClean kits. The five extraction methods yielded DNA with various levels of detection for the three swab materials. For polyester swabs, MagNA Pure Compact and the MasterPure kit performed best, with a limit of detection of 10^4 CFU/ml for both, compared to 10^5 CFU/ml for the IT 1-2-3, QIAamp, and UltraClean kits. Since a 5-μl volume of sample extract was used for the PCRs, this would translate to 50 CFU per PCR for MagNA Pure Compact and the MasterPure kit and 500 CFU per PCR for the IT 1-2-3, QIAamp, and UltraClean kits. For polyurethane foam swabs, the MasterPure kit yielded DNA with the best level of detection by real-time PCR, followed by MagNA Pure Compact and then the IT 1-2-3 and UltraClean kits, which yielded equivalent results. The QIAamp kit yielded DNA with the poorest level of detection from polyurethane foam swabs (10^6 CFU/ml, 5,000 CFU/reaction). For rayon swabs, MagNA Pure Compact and the UltraClean kit performed best (10^4 CFU/ml, 50 CFU/reaction), followed by the MasterPure and QIAamp kits, with the IT 1-2-3 kit providing DNA with the least sensitivity (10^6 CFU/ml, 5,000 CFU/reaction). The differences in mean C_T values for DNA prepared from swab samples with the five extraction methods were found to be significant by one-way ANOVA ($P < 0.05$; $n = 45$). Pairwise comparisons indicated significant differences in mean C_T values for MagNA Pure Compact and the MasterPure kit versus the IT 1-2-3, QIAamp, and UltraClean kits ($P < 0.05$; $n = 45$). There was no PCR inhibition observed for DNA extracts from any of the swab materials, as measured by the IPC real-time PCR assay (data not shown).

Comparison of costs, processing times, and required materials. Table 5 shows comparisons of costs, processing times, recovery volumes, and required materials for the automated and manual DNA extraction methods. Of the six extraction methods, the MasterPure kit was the least expensive on a cost per extraction basis ($1.44). The costs per extraction for the UltraClean and QIAamp kits were comparable, at $1.90 and $2.11, respectively. Of the automated methods, MagNA Pure Compact was the most expensive, at $6.88 per reaction, while the IT 1-2-3 kit was the most expensive of the manual methods

TABLE 3. Comparison of recovery and purity of DNA from *Brucella* spp. by automated and manual extraction methods

Extraction method	Value for[a]:					
	B. abortus		*B. melitensis*		*B. suis*	
	Concn	Ratio	Concn	Ratio	Concn	Ratio
MagNA Pure Compact	3.69	1.59	3.42	1.72	2.95	1.88
MagNA Pure LC	2.11	1.64	2.68	1.69	2.42	1.64
IT 1-2-3	1.90	1.51	1.68	1.47	2.10	1.26
MasterPure	6.41	1.72	6.34	1.72	5.09	1.59
QIAamp	1.87	1.56	1.97	1.50	1.90	1.69
UltraClean	3.76	1.65	3.51	1.71	3.45	1.75

[a] DNA concentrations (ng/μl) were calculated from mean A_{260} measurements for triplicate sample extracts at a concentration of 10^6 CFU/ml. A 2-μl sample was used to quantify each sample. Mean A_{260}/A_{280} ratios were determined for triplicate sample extracts prepared from *Brucella* spp. at concentrations ranging from 10^6 to 10^0 CFU/ml.

TABLE 4. Real-time PCR limit of detection by the use of DNA extracted from spiked swabs

Extraction method[a]	LOD using DNA from indicated swab[b]		
	Polyester	Polyurethane foam	Rayon
MagNA Pure Compact	10^4 (50)	10^5 (500)	10^4 (50)
IT 1-2-3	10^5 (500)	10^5 (500)	10^6 (5,000)
MasterPure	10^4 (50)	10^4 (50)	10^5 (500)
QIAamp	10^5 (500)	10^6 (5,000)	10^5 (500)
UltraClean	10^5 (500)	10^5 (500)	10^4 (50)

[a] Extraction methods were performed on samples recovered from triplicate swabs spiked with 10-fold dilutions of *B. melitensis* at a starting concentration of 10^7 CFU/ml.

[b] The limit of detection (LOD) was determined to be the lowest concentration at which three out of three replicates produced a positive result (CFU/ml [CFU/reaction]).

TABLE 5. Comparison of costs, processing times, sample volumes, and required materials of automated and manual DNA extraction methods

Extraction method	Cost/extraction (U.S. dollars)[a]	Time (h:min)[b]	Recovery vol (μl)	Required reagents	Additional equipment
MagNA Pure Compact	6.88	1:48[c]	100	None	None
MagNA Pure LC	1.97	2:00[c]	100	None	Consumables
IT 1-2-3	3.83	1:05	100	None	Vortex adapter
MasterPure	1.44	2:25	50[d]	100% Isopropanol, 75% ethanol	None
QIAamp	2.11	1:34	200	100% Ethanol	None
UltraClean	1.90	2:05	50	None	Vortex adapter

[a] The cost per extraction was calculated by dividing the cost of each DNA extraction kit, based on the manufacturer's list price in early 2009, by the number of samples that could be processed by each kit.
[b] The total processing time was determined for a 21-sample run. Timing began with the addition of the first reagent and concluded with the recovery of DNA. For automated methods, timing began at the start of DNA extraction on the instrument platforms.
[c] Processing time included a 30-min incubation for performing an external lysis protocol.
[d] The elution volume for this kit was increased from 35 μl (manufacturer's recommendation) to 50 μl to obtain replicates sufficient for statistical analyses.

($3.83). The IT 1-2-3 kit required the least amount of processing time (1 h 5 min), whereas the MasterPure kit required the longest processing time (2 h 25 min). The recovery volumes for sample extracts ranged from 50 μl to 200 μl, with the QIAamp kit yielding the greatest volume and the MasterPure and UltraClean kits producing the smallest volumes. Both the QIAamp and MasterPure kits required the purchase of additional reagents, while MagNA Pure LC, the IT 1-2-3 kit, and the UltraClean kit required additional equipment or consumables.

DISCUSSION

In recent years, molecular diagnostics have become routine in clinical laboratories (31, 32); thus, this study assessed the performance of DNA extraction methods for use in real-time PCR diagnostic assays for *Brucella* spp. It is widely accepted that DNA extraction methods can influence the sensitivity of molecular diagnostic tests at the levels of DNA yield, purity, and damage (28). The results of this study showed that the MasterPure kit resulted in the best limit of detection for the three pathogenic *Brucella* spp. These findings were consistent with a study conducted by Rantakokko-Jalava and Jalava (28), which also reported that the MasterPure kit resulted in the lowest PCR detection level from bacterial suspensions. Factors which likely contributed to this result are the proteinase K lysis procedure and the RNase A treatment used with this kit. Of the six extraction methods evaluated, the MasterPure kit used the longest incubation times, which may have allowed for more-efficient cell lysis and removal of contaminating RNA. In addition, the MasterPure kit used a relatively low resuspension volume for sample extracts, which likely resulted in the higher DNA concentrations obtained with this kit.

Automated processing methods offer several advantages over manual methods, including less hands-on processing time, increased throughput capacity, and less technician-dependent variability (16). The results for automated DNA extraction methods indicated that MagNA Pure Compact was optimal for the recovery of DNA from *Brucella* spp. MagNA Pure Compact also yielded the second-best real-time PCR limit of detection of the six methods evaluated. MagNA Pure LC, however, resulted in the poorest levels of detection by real-time PCR. The results for MagNA Pure LC were consistent with reports for both viruses and bacteria. Schuurman et al. (32) compared automated and manual DNA extraction methods for the detection of viral DNA and reported that MagNA Pure LC resulted in reduced PCR sensitivity. Similarly, a comparative evaluation of manual, semiautomated, and automated DNA extraction methods demonstrated significantly decreased sensitivity for extracts of *Salmonella enterica* prepared with MagNA Pure LC (31). Additionally, Knepp et al. (16) reported that MagNA Pure LC resulted in decreased sensitivity for viral RNA in a comparison of automated and manual nucleic acid extraction methods. Although MagNA Pure LC offers the advantage of increased throughput capacity over manual extraction methods, these findings suggest that other DNA extraction methods should be considered in cases where optimal PCR sensitivity is important.

Many factors can influence the sensitivity of real-time PCR assays, including DNA purity from PCR inhibitors, DNA yield, and DNA damage. The results of this study indicated that overall DNA purity did not greatly influence the levels of detection for *Brucella* spp., as there was no apparent correlation between A_{260}/A_{280} ratios and the real-time PCR results. Furthermore, as determined by the IPC assay, there was no evidence of PCR inhibition in DNA extracts prepared by any method used in this study. In contrast, DNA concentration had some influence on the PCR results as the MasterPure kit, which yielded the highest concentrations of DNA, resulted in the best levels of detection by real-time PCR. This observation held true for all methods except MagNA Pure LC, which did not yield the lowest DNA concentrations, yet yielded the poorest levels of detection. These findings indicate that no one factor can be attributed to the PCR results obtained in this study.

It has been reported that sample matrices can influence the efficiency of DNA extraction methods and subsequently affect the results of real-time PCR assays (6). This study evaluated DNA extraction methods for the recovery of *Brucella* from swabs, which are among the most common specimen types submitted to diagnostic laboratories (15, 18). Of the three swab materials, polyester swabs resulted in DNA with the best levels of detection by real-time PCR, while polyurethane foam and rayon swabs yielded comparable results. However, the analyses were performed independent of extraction methods and are therefore insufficient to recommend polyester as a superior

swab material. Regarding the performance of the DNA extraction methods for spiked swab samples, the MasterPure kit and MagNA Pure Compact yielded DNA with significantly better levels of detection. These findings suggest that either of the two DNA extraction methods is optimal for the recovery and subsequent detection of *Brucella* DNA by real-time PCR.

One goal of this study was to compare several criteria which laboratories may wish to consider when selecting a suitable commercial DNA extraction method. In regard to reagents and supplies, none of the DNA extraction methods required reagents or equipment uncommon in clinical and diagnostic laboratories. In addition, each of the DNA extraction methods offers unique features. Both MagNA Pure Compact and MagNA Pure LC offer all of the advantages of automated sample processing, though the MagNA Pure Compact kit yielded far more-optimal real-time PCR results. Of the manual DNA extraction methods, the IT 1-2-3 kit required the least amount of processing time, which may be important for laboratories that process large numbers of specimens or require rapid time to results. The MasterPure kit was the least expensive and did not require the purchase of additional equipment, which may be important for laboratories for which the cost of DNA extraction kits is an issue. Likewise, the QIAamp kit was relatively inexpensive and did not require the purchase of additional equipment. In addition, the QIAamp kit produced larger sample extract volumes, which may be important for laboratories that perform multiple molecular diagnostic tests. The UltraClean kit was also relatively inexpensive, and it required a moderate processing time in comparison to the other extraction kits.

Given that *Brucella* spp. remain among the most commonly reported causes of laboratory-acquired infections, safety is an important consideration for laboratory personnel who test specimens for *Brucella* spp. For the routine processing of clinical specimens, biosafety level 2 practices within a biological safety cabinet are recommended; however, biosafety level 3 practices are recommended when working with pathogenic cultures of *Brucella* spp. (3). Therefore, this study assessed the ability of the six DNA extraction methods to kill or inactivate virulent *Brucella* spp. The results showed that all of the DNA extraction methods efficiently inactivated *Brucella* spp. at concentrations of $\leq 10^6$ CFU/ml. The results were not surprising as the lysis procedures used in the extraction protocols employed either chemical, mechanical, or heat inactivation, or a combination thereof, all of which are established methods for the inactivation of gram-negative bacteria (20). The findings in this report may also be applicable to other gram-negative bacteria; however, these studies should be performed for certainty.

All of the methods evaluated in this study offer the advantage of safety with regard to processing *Brucella* spp. for subsequent detection using molecular diagnostics. However, the MasterPure kit and MagNA Pure Compact offer clinical and diagnostic laboratories the option of selecting either an automated or manual DNA extraction method for the recovery of *Brucella* DNA with optimal PCR sensitivity.

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B. abortus, *B. melitensis*, and *B. suis* are select agents and their possession, use, and transfer are regulated by the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service. The select agent regulations have mandatory reporting requirements for identification of select agents in diagnostic specimens.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry. Names of vendors or manufacturers are provided as examples of available product sources; inclusion does not imply endorsement of the vendors, manufacturers, or products by the Centers for Disease Control and Prevention or the U.S. Department of Health and Human Services.

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