



A QIAGEN / BD Company



PAXgene[®]
Blood DNA Tube

I. Intended Use

The PAXgene Blood DNA Tube is intended to collect, anticoagulate, stabilize, transport, and store a venous whole blood sample for preparation of human DNA for use with molecular diagnostic test methods that require DNA.

The performance characteristics of this device have not been established for molecular diagnostic assays in general. Users must validate use of product for their specific molecular diagnostic assay.

II. Summary and Explanation

The PAXgene Blood DNA Tube provides a means for the collection of whole blood for the isolation of genomic DNA in a closed, evacuated system. Blood is collected under a standard phlebotomy protocol into an evacuated tube that contains K₂EDTA additive. Complete isolation of DNA is carried out using automated methods such as magnetic bead- or silica membrane-based technologies.

Product Features

- Plastic, evacuated 13 × 75 mm tube with BD Vacutainer® technology and BD Hemogard™ closure
- Nominal draw volume of 2.5 ml with fill indicator
- Unique, 2D barcode on each tube for specimen identification
- Blue stopper with clear BD Hemogard shield to clearly differentiate tube as a DNA testing tube
- DNA concentration, purity and performance in a molecular diagnostic test have been determined on DNA from blood collected in PAXgene Blood DNA Tubes and stored under the following conditions:
 - 14 days at room temperature (18–25°C)
 - 28 days at refrigerated temperature (2–8°C)
 - 3 days at 35°C
 - 52 weeks at –20°C*
 - 3 freeze-thaw cycles*
- Compatible with automated sample preparation methods
- Compatible with magnetic bead- and silica membrane-based technologies.
- The PAXgene Blood DNA Tube is intended for in vitro diagnostic use.

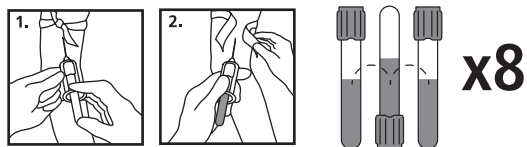
* DNA concentrations in eluates extracted from blood specimens which have undergone 1 freeze-thaw cycle have been shown to fall by more than 25%, with subsequent freeze-thaw cycles not seen to further reduce DNA yield.

III. Specimen Collection and Preparation for Analysis

A. Required Blood Collection Accessories (Not included with the PAXgene Blood DNA Tube)

1. Blood collection device such as the BD Vacutainer Eclipse™ Blood Collection Needle or the BD Vacutainer Safety-Lok™ Blood Collection Set. See Ordering Information.
2. A BD Vacutainer Needle Holder must be used to ensure proper function. See Ordering Information.
3. Labels for positive patient specimen identification, if required.
4. Alcohol swab for cleansing site
5. Dry sterile gauze
6. Tourniquet
7. Needle disposal container for used needle or needle/holder combination

B. Procedure for Specimen Collection



General Instructions

WEAR GLOVES DURING VENIPUNCTURE AND WHEN HANDLING BLOOD COLLECTION TUBES TO MINIMIZE EXPOSURE HAZARD.

1. Using a blood collection set and a tube holder, collect blood into the PAXgene Blood DNA Tube using your institution's recommended procedure for standard venipuncture technique.
2. Immediately after blood collection, gently invert the PAXgene Blood DNA Tube 8 times.
3. Store the filled PAXgene Blood DNA Tube at room temperature (18–25°C) or refrigerate at 2–8°C. See Performance Characteristics for DNA stability in blood samples at 18–25°C, 2–8°C, 35°C, –20°C, or after freeze-thaw cycles.

See Procedure for Freezing and Thawing Specimens (Section C.) for details if tubes are to be frozen for shipping or storage.

Recommended Order of Draw

1. Tubes for sterile samples
2. Tubes for coagulation studies (e.g., citrate)
3. BD SST™, BD SST™ II Advance and serum tubes
4. PAXgene Blood DNA Tubes

The following techniques shall be used to prevent possible backflow:

- a. Place patient's arm in a downward position.
- b. Hold tube with the stopper uppermost.
- c. Release tourniquet as soon as blood starts to flow into tube.

C. Procedure for Freezing and Thawing Specimens Collected in the PAXgene Blood DNA Tubes

1. Stand the PAXgene Blood DNA Tube upright in a wire rack. Do not freeze tubes upright in a Styrofoam™ tray as this may cause the tubes to crack.
2. Store the PAXgene Blood DNA Tubes at -20°C.
3. Thaw the PAXgene Blood DNA Tubes in a wire rack at room temperature (18–25°C) for approximately two hours. Do not thaw the PAXgene Blood DNA Tubes at temperatures above 25°C.
4. Carefully invert the thawed PAXgene Blood DNA Tubes 10 times.

Note: Frozen PAXgene Blood DNA Tubes are subject to breakage upon impact. To reduce the risk of breakage during shipment, frozen tubes should be treated in the same manner as glass tubes. Users must validate their own freezing and shipping protocol for PAXgene Blood DNA Tubes.

D. Procedure for Specimen Preparation for Analysis

Blood samples should be processed in accordance with the instructions provided with the DNA sample preparation kit. For examples, see Ordering Information.

IV. Performance Characteristics

After blood is introduced into the tube, the DNA is suitable for DNA testing for 14 days at 18–25°C (Figures 1 and 3), 28 days at 2–8°C (Figures 2 and 4), for a minimum of 52 weeks at -20°C (Figure 5), after 3 freeze-thaw cycles (Figure 6), or up to 3 days at 35°C (Figure 7).

Stability of blood stored in the tube was tested for DNA concentration and purity, as well as HLA Assay 2 performance. The data supports storage of blood in the tube for the following conditions:

Table 1: Whole blood storage conditions and assay results

Storage Time	Storage Temperature	Sample Size	Concentration (ng DNA / µl eluate)	Purity (A ₂₆₀ /A ₂₈₀)	Assay Concordance	95% CI Lower Bound
0, 3, 7, 14 days	18–25°C	12	≥ 17.5	1.8–1.9	100% (48/48)	92.6%
0, 14 days	18–25°C	60	≥ 16.7	1.7–1.9	100% (120/120)	96.9%
0, 7, 14, 21, 28 days	2–8°C	12	≥ 13.4	1.8–1.9	100% (36/36)	90.4%
0, 28 days	2–8°C	60	≥ 16.3	1.7–1.9	100% (120/120)	96.9%
0, 1, 6, 12 months	-20°C	12	≥ 15.3	1.8–1.9	100% (48/48)	92.6%
1, 2, 3 freeze-thaw cycles	-20°C / 18–25°C	12	≥ 16.1	1.7–1.9	100% (24/24)	86.2%
1, 2, 3 freeze-thaw cycles	-20°C / 18–25°C	60	≥ 13.1	1.7–1.9	N/A	N/A
6 hours, 1, 2, 3 days	35°C	12	≥ 14.1	1.7–1.9	100% (60/60)	94.0%

DNA concentration and purity were assessed for the PAXgene Blood DNA Tube using a commercially available, automated magnetic bead based DNA extraction kit (elution volume: 200 µl). DNA concentration was ≥ 13.1 ng/µl and DNA purity was between 1.7–1.9 for all samples.

DNA Concentration for Blood Samples Stored at 18–25°C: Day 0 vs. Day 14

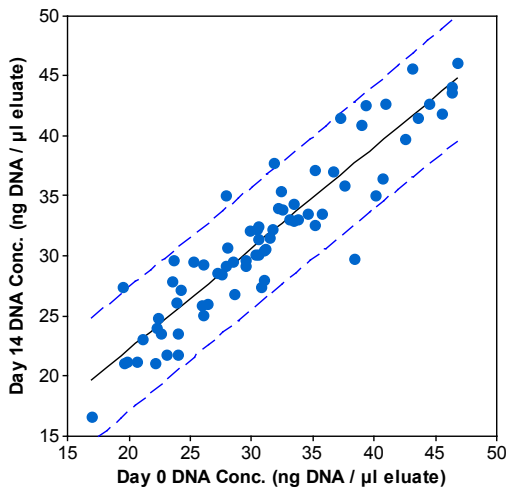


Figure 1

Blood was drawn from each of 72 consented subjects ≥ 18 years of age into two PAXgene Blood DNA Tubes. One tube from each subject was processed within two hours of collection (Day 0) and the other tube was stored at 18–25°C for 14 days after which time the DNA was extracted. Total DNA was purified using a commercially available, automated magnetic bead based DNA extraction kit (elution volume: 200 µl). Regression line (Day 14 Concentration = 5.39 + 0.84 Day 0 Concentration, R² = 86.0%) and 95% prediction interval (dashed lines) shown.

DNA Concentration for Blood Samples Stored at 2–8°C: Day 0 vs. Day 28

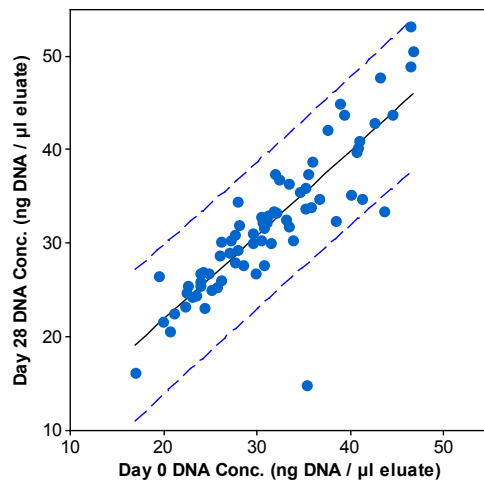


Figure 2

Blood was drawn from each of 72 consented subjects ≥ 18 years of age into two PAXgene Blood DNA Tubes. One tube from each subject was processed within two hours of collection (Day 0) and the other tube was stored at 2–8°C for 28 days after which time the DNA was extracted. Total DNA was purified using a commercially available, automated magnetic bead based DNA extraction kit (elution volume: 200 µl). Regression line (Day 28 Concentration = 3.81 + 0.90 Day 0 Concentration, R² = 74.0%) and 95% prediction interval (dashed lines) shown.

DNA Concentration for Blood Samples Stored in the PAXgene Blood DNA Tube

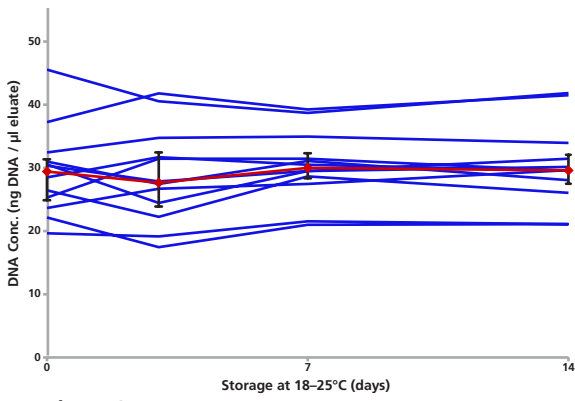


Figure 3

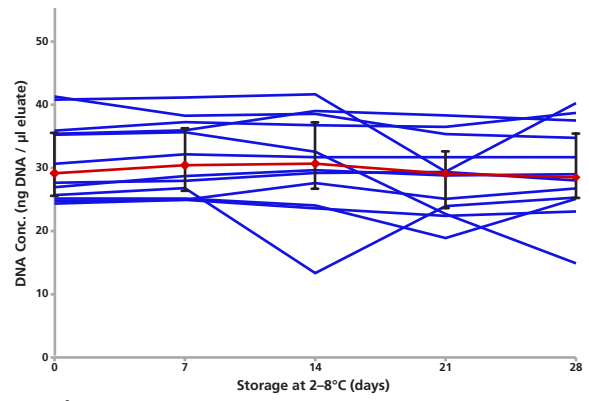


Figure 4

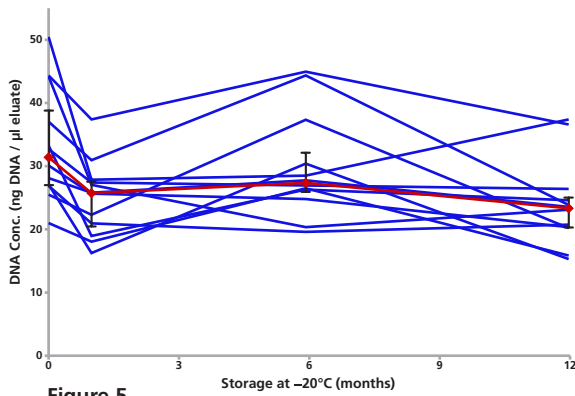


Figure 5

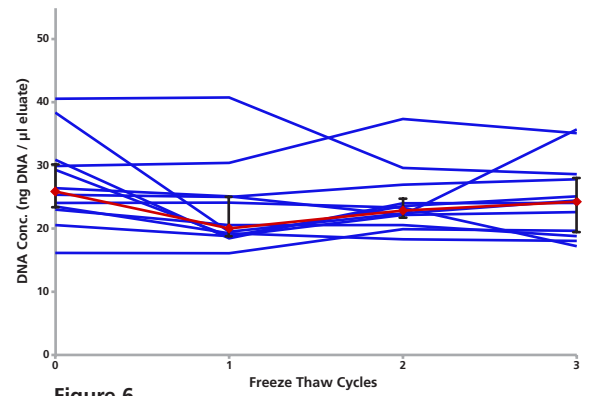


Figure 6

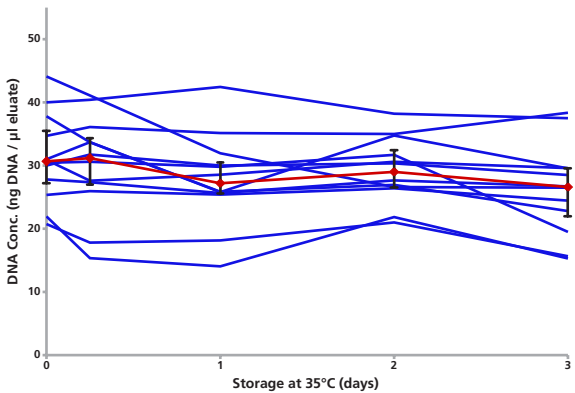


Figure 7

Figures 3-7

For each study, blood was drawn from each of 12 consented subjects ≥ 18 years of age into replicate PAXgene Blood DNA Tubes. One tube from each subject was processed within two hours of collection (Day 0) and the other tubes were stored at 18-25°C (Figure 3), 2-8°C (Figure 4), -20°C (Figure 5), subjected to -20°C freeze-thaw cycles (Figure 6), or 35°C (Figure 7). Total DNA was purified using a commercially available, automated magnetic bead based DNA extraction kit (elution volume: 200 μ l). Each blue line represents the measurements of an individual subject. Means of all subjects are shown as red diamonds with error bars denoting the 25% and 75% quartiles.

DNA Eluate Concentrations and Purity using an Automated, Magnetic Bead-Based DNA Purification System

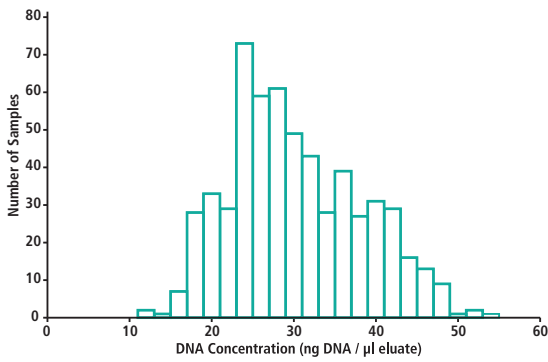


Figure 8

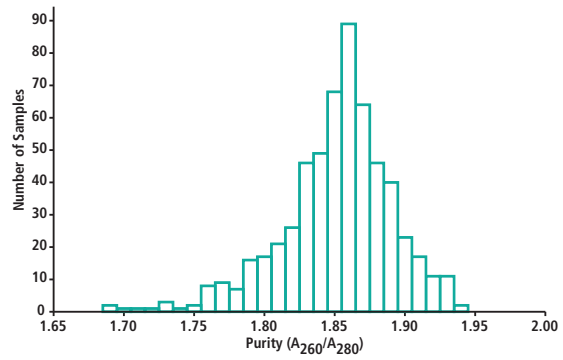


Figure 9

Blood was drawn from a donor pool of approximately 200 consented subjects ≥ 18 years of age into PAXgene Blood DNA Tubes. Tubes were processed within 24 hours at room temperature. Total DNA was purified from 581 specimens using a commercially available, automated magnetic bead based DNA extraction kit (elution volume: 200 μ l).

Table 2: Performance testing summary (magnetic bead-based DNA purification)

	Yield (μ g DNA / 200 μ l blood)	Concentration (ng DNA / μ l eluate)	Purity (A_{260}/A_{280})
n	581	581	581
Mean \pm SD	6.05 \pm 1.61	30.2 \pm 8.0	1.85 \pm 0.04
Median	5.77	28.9	1.86
Interquartile range	4.88–7.22	24.4–36.1	1.83–1.88
Range	2.43–10.79	12.2–54.0	1.69–1.94
95% of samples	≥ 3.64	≥ 18.2	1.75–1.93

DNA Eluate Concentrations and Purity using an Automated, Silica Membrane-Based DNA Purification System

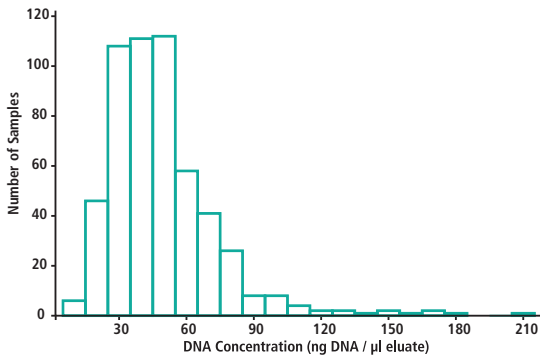


Figure 10

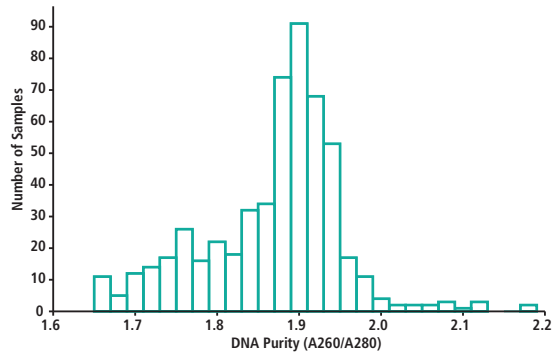


Figure 11

Blood was drawn from 152 consented subjects ≥ 18 years of age into PAXgene Blood DNA Tubes. Tubes were stored at room temperature for ≤ 14 days. Total DNA was purified from 540 specimens using a commercially available, automated silica membrane based DNA extraction kit (elution volume: 100 μ l).

Table 3: Performance testing summary (silica membrane-based DNA purification)

	Yield (μ g DNA / 200 μ l blood)	Concentration (ng DNA / μ l eluate)	Purity (A_{260}/A_{280})
n	540	540	540
Mean \pm SD	4.89 \pm 2.48	48.85 \pm 24.75	1.86 \pm 0.08
Median	4.49	44.90	1.88
Interquartile range	3.27–5.71	32.73–57.10	1.81–1.92
Range	0.75–21.1	7.46–211.10	1.65–2.19
95% of samples	≥ 1.86	≥ 18.56	1.67–2.06

Performance with Molecular Diagnostic Test Methods

Evaluations of the PAXgene Blood DNA Tube have been performed for selected assays on certain instrument platforms. See Table 4 for sample preparation, instrument, and assay information.

Table 4: Assay and DNA Sample Preparation Information:

Assay	Cystic Fibrosis Assay	HLA Assay 1	Thrombophilia Assay	HLA Assay 2	HLA Assay 3
Assay instrument technology	Multiplex fluorescent microsphere based flow cytometry	Multiplex fluorescent microsphere based flow cytometry	Electrochemical detection based DNA microarray	N/A, gel-based readout	N/A, gel-based readout
DNA isolation kit and instrument technology	Silica membrane	Silica membrane	Silica membrane	Magnetic bead	Magnetic bead and silica membrane

The performance of the PAXgene Blood DNA Tube was assessed relative to an EDTA tube control using FDA cleared molecular diagnostic assays. Assays were evaluated at either 1 or 3 sites. See Table 5 and Table 6 for testing results:

Table 5: Test Results by Site:

Site	Assay	Samples tested	Correct calls	Incorrect calls	No calls	% Correct calls	95% CI lower bound
Site A	CF Assay	40	40	0	0	100%	91.2%
	HLA Assay 1	40	40	0	0	100%	91.2%
Site B	CF Assay, After Retest [†]	37	36	0	1	97.3%	86.2%
	HLA Assay 1 [*]	40	40	0	0	100%	91.2%
Site C	CF Assay	40	40	0	0	100%	91.2%
	HLA Assay 1, After Retest ^{**}	40	40	0	0	100%	91.2%
Site D	Thrombophilia	80	80	0	0	100%	95.2%
Site E	HLA Assay 2, After Retest [§]	698	698	0	0	100%	99.5%
	HLA Assay 3	100	100	0	0	100%	96.3%

* In addition to the two-field concordance presented in the table, probe hit patterns were analyzed and a total of 7 probes out of 14,400 (200 comparisons × 72 probes) were found to be discordant. The overall probe concordance was 99.95% with a 95% CI lower bound of 99.9%.

† CF Assay, after retest, includes 1 sample from Site B showing a result of “No Call” that was not retested. Three previous runs at Site B included up to 38 samples showing a result of “No Call” due to a degraded enzyme in the CF Assay Kit. Run 4 used a new enzyme to perform the test at Site B. The results exclude 3 subjects from Site B where the assay was not repeated for 3 evaluation tubes.

‡ HLA Assay 1, after retest, includes 4 samples from Site C that were re-extracted and retested due to a labeling error.

§ HLA Assay 2, after retest, includes 2 repeat testing samples due to labeling errors and removes 12 samples (3 concordant with previous results, 9 discordant with previous results due to labeling errors) that could not be retested.

Table 6: Test Results by Study:

Objective	Sites	Assay tested	Samples calls	Correct calls	Incorrect calls	No calls	% Correct calls	95% CI lower bound
Site-to-site reproducibility	A, B, C	CF Assay	20	20	0	0	100%	N/A
	A, B, C	HLA Assay 1 [*]	20	20	0	0	100%	N/A
Lot-to-lot variation	A	CF Assay	20	20	0	0	100%	N/A
	A	HLA Assay 1	20	20	0	0	100%	N/A
Tube performance	A, B, C	CF Assay, After Retest [†]	117	116	0	1	99.1%	95.3%
	A, B, C	HLA Assay 1, After Retest ^{**}	120	120	0	0	100%	96.9%
	D	Thrombophilia Assay	80	80	0	0	100%	95.4%
	E	HLA Assay 2, After Retest [§]	698	698	0	0	100%	99.5%
Interference	E	HLA Assay 3	100	100	0	0	100%	96.3%

* In addition to the two-field concordance presented in the table, probe hit patterns were analyzed and a total of 7 probes out of 14,400 (200 comparisons × 72 probes) were found to be discordant. The overall probe concordance was 99.95% with a 95% CI lower bound of 99.9%.

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Interfering substances

Potentially interfering substances were added separately to the PAXgene Blood DNA Tube. The following substances were evaluated:

Table 7. Interfering substances – Concentrations tested

Interfering substance	Hemoglobin	Bilirubin	Triglycerides	Albumin
Concentration	200 g/L*	200 mg/L†	18.2 g/L†	27.4 g/L†

* Total concentration includes endogenous and added hemoglobin

† Concentration of interferent added to sample

DNA concentration and purity were assessed for the PAXgene Blood DNA Tube using both a commercially available, automated magnetic bead based DNA extraction kit (elution volume: 200 µl) and a commercially available, automated silica membrane based DNA extraction kit (elution volume: 100 µl). DNA concentration was ≥ 43.6 ng/µl for magnetic bead based DNA extraction kit samples and ≥ 24.0 ng/µl for silica membrane based DNA extraction kit samples. DNA purity was between 1.7–1.9 for all samples.

The addition of these substances did not have an effect on the FDA cleared assay performance (See Table 6). All samples were concordant with a PAXgene Blood DNA Tube from the same subject without the added substances.

V. Limitations

1. The quantity of blood drawn should be approximately 2.5 ml per PAXgene Blood DNA Tube, but this volume may vary with altitude, ambient temperature, barometric pressure, tube age, venous pressure, and filling technique. Please note that the PAXgene Blood DNA Tube may fill more slowly than other blood collection tubes of the same size but with larger draw volumes.
2. DNA yields depend on the number of nucleated cells in the sample, the quality of the specimen, and the method used for isolation of DNA. Using smaller elution buffer volumes with silica membrane columns or magnetic beads increases the final DNA concentration in the eluate but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application.
3. DNA concentrations in eluates extracted from blood specimens which have undergone 1 freeze-thaw cycle have been shown to fall by more than 25%, with subsequent freeze-thaw cycles not seen to further reduce DNA yield¹. For highest yield, DNA should be extracted from blood collected in the PAXgene Blood DNA Tube prior to freezing. Up to 3 freeze-thaw cycles does not negatively affect DNA quality or performance in molecular diagnostic test methods.
4. The PAXgene Blood DNA Tube is not intended for blood donor screening.
5. The PAXgene Blood DNA Tube has not been validated for the following technologies or samples: next generation sequencing, cytogenetic arrays, FISH assays, somatic mutations such as might be tested for hematological oncology purposes.
6. Endotoxin not controlled. Blood and blood components collected and processed in the tube are not intended for infusion or introduction into the human body.
7. The PAXgene Blood DNA Tube has not been validated for the collection, stabilization, storage or extraction of microbial DNA from human whole blood samples, and has not been cleared or approved for laboratory tests that detect microbial nucleic acids.
8. The PAXgene Blood DNA Tube has not been validated with DNA extraction methods involving manual precipitation.

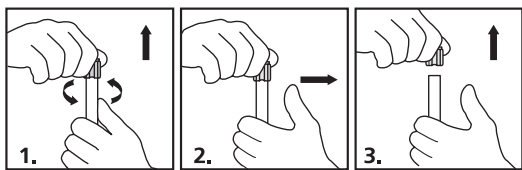
VI. Precautions

1. Practice universal precautions. Use gloves, gowns, eye protection, other personal protective equipment, and engineering controls to protect from blood splatter, blood leakage, and potential exposure to bloodborne pathogens.
2. Handle all biologic samples and blood collection devices according to the policies and procedures of your facility. Obtain appropriate medical attention in the event of any exposure to biologic samples (for example, through a puncture injury), since they may transmit viral hepatitis, HIV (AIDS), or other infectious diseases. Utilize any built-in needle protector, if the blood collection device provides one. PreAnalytiX does not recommend reshielding used needles. However, the policies and procedures of your facility may differ and must always be followed.
3. Discard all blood collection tubes in biohazard containers approved for their disposal.
4. Do not re-use the PAXgene Blood DNA Tubes.
5. Do not use the PAXgene Blood DNA Tubes after the expiration date printed on the tube label.
6. Do not centrifuge the PAXgene Blood DNA Tube.
7. Do not transfer a specimen from a syringe into a tube.

VII. Storage

1. Store the unused PAXgene Blood DNA Tubes at 18–25°C. Stability studies have shown the product to be stable for up to 7 days at 40°C. Do not use tubes after their expiration date.

VIII. Instructions for Removal of BD Hemogard Closure



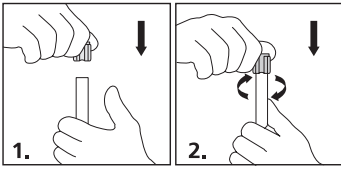
1. Grasp the PAXgene Blood DNA Tube with one hand, placing the thumb under the BD Hemogard Closure. (For added stability, place arm on solid surface). With the other hand, twist the BD Hemogard Closure while simultaneously pushing up with the thumb of the other hand ONLY UNTIL THE TUBE STOPPER IS LOOSENED.

2. Move thumb away before lifting closure. DO NOT use thumb to push closure off tube. If the tube contains blood, an exposure hazard exists. To help prevent injury during closure removal, it is important that the thumb used to push upward on the closure be removed from contact with the tube as soon as the BD Hemogard Closure is loosened.

3. Lift closure off tube. In the unlikely event of the plastic shield separating from the rubber stopper, DO NOT REASSEMBLE CLOSURE. Carefully remove rubber stopper from tube.

¹ Ross, KS, Haites NE, and Kelly KF. "Repeated freezing and thawing of peripheral blood and DNA in suspension: effects on DNA yield and integrity." *Journal of Medical Genetics* (1990); 27(9): 569-70.

IX. Instructions for Reinsertion of BD Hemogard Closure



1. Replace closure over tube.
2. Twist and push down firmly until stopper is fully resealed. Complete reinsertion of the stopper is necessary for the closure to remain securely on the tube during handling.

Technical Assistance

If you have any questions regarding the PAXgene Blood DNA Tube, contact one of the BD Technical Services Departments listed in the BD - Customer Service section.

Ordering Information

PAXgene Products

PAXgene Blood DNA Tube / 761165

Content: 1,000 blood collection tubes

2.5 ml • 13 x 75 mm • K₂EDTA additive • Plus • BD Hemogard

100 tubes/shelf carton • 1,000 tubes/case

BD Products*

BD Vacutainer Push Button Blood Collection Set / 367344 NA

Content: 21G 3/4 inch (0.8 x 19 mm) needle, 12 inch (305 mm) tubing with luer adapter. 50/box, 200/case

BD Vacutainer Push Button Blood Collection Set with Pre-Attached Holder / 367352 NA

Content: 21G 3/4 inch (0.8 x 19 mm) needle, 12 inch (305 mm) tubing with holder. 20/box, 100/case

BD Vacutainer Safety-Lok Blood Collection Set / 367281 US / 367286 CE

Content: 21G 3/4 inch (0.8 x 19 mm) needle, 12 inch (305 mm) tubing with luer adapter. 50/box, 200/case

BD Vacutainer Eclipse Blood Collection Needle / 368607 NA / 368609 CE

Content: 21G 1 1/4 inch (0.8 x 32 mm) needle, 48/box, 480/case

BD Vacutainer Eclipse Blood Collection Needle with Pre-Attached Holder / 368650

Content: 21G 1 1/4 inch (0.8 x 32 mm) needle, 100/case

BD Vacutainer Eclipse Signal™ Blood Collection Needle / 368835 CE

Content: 21G 1 1/4 inch (0.8 x 32 mm) needle, 50/box, 400/case

BD Vacutainer One Use Holder / 364815

Content: Case only for 13 mm and 16 mm diameter 1000/case

* These catalog numbers represent typical products that can be used with the PAXgene Blood DNA Tube.

To order BD Blood Collection Accessories:

Go to: <http://www.bd.com/support/contact/international.asp>

• Select country • Scroll down for BD Diagnostics - Preanalytical Systems • Dial number

QIAGEN Products*

QIAGEN QIASymphony DSP DNA Kit / 937255 Midi (96) / 937236 Mini (192)

Content: Reagent cartridge, enzyme rack, piercing lid, buffer ATE, reuse seal set

QIAGEN QIAamp DSP DNA Blood Mini Kit / 61104 (50)

Content: QIAamp Mini spin columns, collection tubes, buffer AL, buffer ATL, buffer AW1, buffer AW2, buffer AE, QIAGEN protease, protease solvent

* These catalog numbers represent typical DNA preparation kits that can be used with the PAXgene Blood DNA Tube.

To order QIAGEN DNA Preparation Kits:

Go to: <http://www.qiagen.com/Products/Ordering-Information/>

BD – Customer Service

Argentina, Uruguay and Paraguay

Orders: 0800.444.5523

E-mail: crc_argentina@bd.com

Australia

Orders: 1.800.656.100

Fax: 1.800.656.110

E-mail: bd_anz@bd.com

Austria

Orders: 43.1.706.36.60

Fax: 43.1.706.36.60-11

E-mail: customerservice.vac.ga@europe.bd.com

Belgium

Orders: 32.53.720.556

Fax: 32.53.720.549

E-mail: orders.be@europe.bd.com

Brazil

Orders: 0800.055.56.54

E-mail: consultoria_vacutainer@bd.com

Canada

Orders: 800.268.5430

Fax: 800.565.0897

E-mail: custsvccan@bd.com

Central and Eastern Europe

Orders: 48.22.377.11.11

Fax: 48.22.377.11.02

Bulgaria orders: info_bulgaria@europe.bd.com

Czech Republic orders: info_czech@europe.bd.com

Croatia orders: info_croatia@europe.bd.com

Hungary orders: info_hungary@europe.bd.com

Poland orders: info_poland@europe.bd.com

Romania orders: info_romania@europe.bd.com

Southeast Europe orders: info_balkan@europe.bd.com

Serbia orders: info_serbia@europe.bd.com

Slovakia orders: info_slovakia@europe.bd.com

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Orders: 33.476.68.94.96

Fax: 33.476.68.36.93

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Germany

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