510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k042613

B. Purpose for Submission:
Clearance of new device

C. Measurand:
Ribonucleic acid (RNA)

D. Type of Test:
Collection, stabilization, and purification of intercellular RNA from whole blood for RT-PCR use in molecular diagnostic testing.

E. Applicant:
PreAnalytiX GmbH

F. Proprietary and Established Names:
PAXgene™ Blood RNA System

G. Regulatory Information:
1. Regulation section:
   21 CFR §866.4070, RNA Preanalytical Systems
2. Classification:
   Class II (de novo)
3. Product code:
   NTW
4. Panel:
   Immunology (82)

H. Intended Use:
1. Intended use(s):
   The PAXgene™ Blood RNA System consists of a blood collection tube (PAXgene™ Blood RNA Tube) and nucleic acid purification kit (PAXgene™ Blood RNA Kit). It is intended for the collection, storage, and transport of blood and stabilization of intracellular RNA in a closed tube and subsequent isolation and purification of host RNA from whole blood for RT-PCR used in molecular diagnostic testing.

   Performance characteristics for the PAXgene™ Blood RNA System have only been established with "cfos and IL1B." The user is responsible for establishing appropriate PAXgene™ Blood RNA System performance characteristics for other target transcripts.

2. Indication(s) for use:
   See intended use above

3. Special conditions for use statement(s):
   For professional use only

4. Special instrument requirements:
   General laboratory equipment (centrifuges, etc.)

I. Device Description:

The PAXgene™ Blood RNA System consists of:

- the PAXgene™ Blood RNA tubes and
- the PAXgene™ Blood RNA kit.

The PAXgene™ Blood RNA tube is of a sterile, plastic, evacuated blood collection tube containing stabilization solution (tetradecyl trimethyl-ammonium oxalate and tartaric acid. These components serve to lyse cells, protect RNA molecules from degradation by ribonucleases (RNases) and prevent induction of gene expression.

The kit consists of 5 aqueous buffer solutions for resuspending, binding, washing, and eluting RNA, RNase-free water, proteinase K, an RNase-Free DNase set, spin columns, microcentrifuge tubes, processing tubes, and secondary blood collection tube closures.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   None
2. Predicate 510(k) number(s):
   None
3. Comparison with predicate:
   Not applicable

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

Blood (2.5 mL) is collected into the PAXgene™ Blood RNA tube by venipuncture. It can be either stored or immediately processed (according to specifications in the labeling). To isolate RNA from the sample, the blood is centrifuged to sediment the nucleic acids and the pellet is resuspended in and washed with RNase-free water. The resulting pellet is dissolved in buffer BR1 and buffer BR2, and proteinase K is added for the enzymatic digestion of proteins. Genomic DNA is sheared by running the sample through a shredder spin column, and ethanol is added to adjust the binding conditions. RNA is selectively bound to the membrane in the PAXgene™ Blood RNA spin column by a short centrifugation. The bound RNA is washed, and residual genomic DNA is removed from the membrane column by treatment of the bound nucleic acid with DNase I. RNA is eluted from the column using the elution buffer

provided.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

CFOS/18S rRNA and IL1B/18S rRNA Q-RT-PCR Duplex assays.

Objective: The sponsor submitted validation and performance data for two **uncleared** Q-RT-PCR assays for the assessment of performance of the PAXgene Blood RNA System in comparison to a K_2EDTA blood collection tube and acid phenol RNA extraction. The assays determine quantitativly the relative level of the c-fos and IL-1ß transcripts compared to 18S rRNA in RNA samples derived from human whole blood. Since CFOS and IL1B offer no diagnostic information, these assays are not intended for diagnostic use. They are ONLY intended for demonstrating that the PAXgene Blood RNA System can be used for RT-PCR.

The validation study uses two types of samples:

K_2EDTA (TOTAL: 250 ml blood):

- 5 donors with 5 tubes per donor
- 10 ml blood per EDTA tube
- RNA was isolated via QIAzol and RNeasy columns, pooled and concentrated via MinElute spin column
- Purified, concentrated RNA was analyzed for yield and purity, aliquoted, and stored at -20 °C.

PAXgene (TOTAL: 960 ml blood):

- 48 donors, 8 tubes per donor (total of **384** PAXgene tubes)
- 2.5 ml blood per PAXgene tube
- tubes were incubated at RT for 2 hrs
- RNA was purified, DNase treated, concentrated via MinElute spin column and pooled.
- Purified, concentrated RNA was analyzed for yield and purity, aliquoted, and stored at -20 °C.

Experimental design of validation experiments:

The sponsor addressed repeatability and reproducibility and defines these terms within the document as follows:

The repeatability of the assay (within-run precision or intra-assay variability), is the **variability of the results** from repeated measurements:

- using the same two source samples (n = 10 for each sample)
- in the same laboratory (same equipment and same experimenter),
- on the same day,
- with the same reagents (identical lots) and

- in the same run.

The repeatability of individual runs is expressed as the weighted mean repeatability used for the calculation of overall precision. The weighted mean repeatability is determined and is included in the overall precision calculation.

The reproducibility of the assay, (robustness, run-to-run precision, or inter-assay variability), is the **variability of results** from repeat measurements

- using the same two source samples
- in different laboratories (different lots, different equipment and different experimenters)
- on different days
- and in different runs.

Therefore, reproducibility investigations involve repeats of the complete experiment in different laboratories. To establish the suitability of the precision for day-to-day work, different lots of reagents are investigated as additional parameters that may have a negative impact on precision. The reproducibility between laboratories is determined, but also not specified in detail, because it is included in the overall precision calculation and therefore will be assessed as its part.

Table 6: Summary of measurement runs for the determination of overall precision

Combi-nation[1)]	Reagent Combination Run No. (k)	Run[2)]	RNA sample[3)]	Replicates per RNA sample	Number of ΔCTvalues per RNA sample	Number of ΔΔCT values per run
A	1	A1	K	10	10	10
			T	10	10	
	2	A2	K	10	10	10
			T	10	10	
	3	A3	K	10	10	10
			T	10	10	
B	4	B1	K	10	10	10
			T	10	10	
	5	B2	K	10	10	10
			T	10	10	
	6	B3	K	10	10	10
			T	10	10	
C	7	C1	K	10	10	10
			T	10	10	
	8	C2	K	10	10	10
			T	10	10	
	9	C3	K	10	10	10
			T	10	10	
Total					180	**90**

[1)] Combination of experimenter, laboratory (equipment), day, TaqMan plate
[2)] Measurement series (run). Established by reagent lots within the combination (A, B, C) determined by reagent lots [3)] K = calibrator RNA, T = test RNA

Note: Under 1, technician-to-technician, lab-to-lab, and day-to-day variability has been combined in this study.

Results: The sponsor performed an appropriate validation of their submitted assay by using 3 different component lots, in the hands of 3 different experimenters, in 3 different laboratories (equipment), on 3 different days. The sponsor provided all raw CT data and demonstrated the performance of their assays within the expected limits.

Precision/Reproducibility of the PAXgene Blood RNA System.

Objective: Determination of the repeatability, reproducibility and reliability of the PAXgene Blood RNA System.

Two experiments were performed:
Experiment 1:

- 14 donors (WBC counts within **4.8 to 11.0 x 10^6** cells/ml blood**)**
- 12 tubes per donor (total **168** PAXgene tubes)
- PAXgene Blood RNA system Lot No. VL1 (ONLY one lot!)
- 3 technicians. Each technician processed 4 tubes per subject (total of 56 tubes per technician) in 3 runs (Run #1: donor 1-3 (12 tubes); Run #2: donor 4-9 (24 tubes); Run #3: donor 10-14 (20 tubes)

RNA yield (absorbance at 260 nm) and **purity** (260nm/280nm absorbance ratio) were determined for each sample.
Repeatibility was determined by evaluating the yield and purity of four samples from one donor processed by one technician.
Reproducibility was determined by evaluating the yield and purity of 12 samples from one donor processed by three technicians. The repeatability and reproducibility was determined separately for each donor because of the differences between individuals.

Experiment 2:

- 30 donors (WBC counts within **4.8 to 11.0 x 10^6** cells/ml blood**)**
- 12 tubes per donor (total **360** PAXgene tubes)
- Blood from filled PAXgene tubes from 3 donors were pooled and realiquoted into empty tubes, generating 10 donor pools with 36 tubes each.
- PAXgene Blood RNA system Lot No. VL1, VL2, VL3
- 3 technicians. Each technician processed 4 tubes per donor pool per day. This was repeated with 2 additional kit lots.

RNA yield (absorbance at 260 nm) and **purity** (260nm/280nm absorbance ratio) were determined for each sample. For this experiment the RNA purity was also evaluated by electropherograms generated by BioAnalyzer. The isolated total RNA was further tested for degradation with the CFOS and IL1B Q-RT-PCR assay.

Results: the sponsor was able to show that the RNA yield and the RNA purity and quality were within the specification ranges. The min/max % CV repeatability for the quadruplicate preparation per donor pool within each lot and user were 3.4 and 28.8, respectively, with an overall % CV of 11.9. The min/max % CVs reproducibility within each user and between all lots were 8.7 and 20.1, respectively, with an overall % CV of 14.9. The min/max % CV reproducibility within all lots and between all users were 8.7 and 23.1, respectively, with an overall % CV of 16.4. The electropherograms demonstrated that the device repeatedly generates high quality total cellular RNA from whole blood. The Q-RT-PCR assay demonstrated that the transcripts CFOS and IL1B can be repeatedly measured within the assays limits utilizing different device lots, at different days, with different users in different laboratories.

RNA Purity, DNA Contamination, RNA Yield.

Objective:
Determination of the **purity of RNA** in samples, the **genomic DNA** (gDNA) in RNA samples and the **nucleic acid yield** from human whole blood specimens taken from normal subject population resulting from collection and processing of human whole blood in the PAXgene RNA Blood System.

Experimental design:

- 10 donors
- 2 tubes per donor (**total 20 PAXgene tubes**) + 1 EDTA tube per donor for WBC count (10 EDTA tubes)
- storage for 24 hrs at 18-22 °C, tubes were processed, RNA isolated
- RNA yield and purity were determined via absorbance measurement at 260 and 280 nm
- Percent gDNA in total nucleic acid preparation was determined using beta-actin PCR

Results: All samples for all parameter fulfilled the acceptance criteria.

RT-PCR Inhibition.

Objective:
Determination of RT-PCR inhibition introduced into RNA isolates by components of the PAXgene Blood RNA Kit

Experimental Design:

- 22 blank eluates form columns
- template RNA for RT-PCR assay was isolated from confluent layer of HeLa cells

- Test samples were formulated each containing 40 ng of HeLa RNA template plus 0, 10, 20, 30, 40% volume of volume of blank eluate
- Template RNA with eluate was tested in duplicate in a one-step ACTB RT-PCR assay

Results: All samples demonstrated that the kit components do not introduce any inhibition for an RT-PCR assay.

b. *Linearity/assay reportable range:*
The linearity and range was determined within 0.2 to 7.8 ng for CFOS and 0.1 to 7.8 ng for IL1B total input nucleic acid per reaction. The total input mass of nucleic acid per reaction can contain up to 25% DNA for CFOS and up to 5% DNA for IL1B without affecting the performance characteristics of the assay.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Stability of PAXgene Tubes:
Objective: Determine the shelf life of the PAXgene Blood RNA Tube.
Determination of physical attributes of the tubes:
- draw volume
- liquid additive volume
- closure performance

Chemical attributes of the liquid reagent:
- pH
- conductivity
- density
- chemical component concentration

Analysis of tube function:
- RNA yield (A260 nm, 95% ≥3 μg/tube)
- Purity (ratio of A260 and A280, between 1.8 – 2.2)
- Relative levels of CFOS and IL1B (CFOS/18S rRNA and IL1B/18S rRNA duplex Q-RT-PCR assay, CFOS within 2.34 CT and IL1B within 1.94 CT).

Blood was collected in PAXgene tubes that had been stored at designated test time points (TTP's). RNA stabilizing properties of the tubes were investigated by preparing purified RNA from blood-filled tubes stored at 18-25°C for 0, 1 and 3 days with the PAXgene blood RNA kit (10 donors x 3 times of storage of blood filled tubes x 2 preparations). All PAXgene Blood RNA kits used in these studies were stored prior to use at 2-8°C to minimize kit aging. IMPORTANT: All claims of stability that are based on data from accelerated stability studies will be confirmed in parallel in real time stability studies. Table 1 shows the number of tubes tested in functional tests at each TTP.

Table 1: Functional Test TTPs and number of tubes scheduled for testing

Stability Conditions	TTPs at this temperature	number of tubes for thistemperature study	total
40°C study (accelerated)	19w, 28w	2 TTP x 60tubes/TTP	120
50°C study (accelerated)	10w, 15w	2 TTP x 60 tubes/TTP	120
25°C study (real time)	0, 6m, 9m, 12m, 13m, 15m, 16m, 18m, 19m	9 TTP x 60 tubes/TTP	810

Results: Accelerated stability studies have been completed at the time of this report. Real time stability studies are ongoing, and only results of 6 months storage at 25°C are presented. The sponsor interprets that the data support a shelf live of 19 months storage at 25 °C.

The physical attributes were evaluated for 3 different lots of tubes. The draw volume, liquid additive volume, and closure performance meet the acceptance criteria.
For the chemical attributes as well as the functional performance it is not clear whether 1 or 3 lots of tubes were used. For the presented data the pH, conductivity, and density as well as the RNA yield, purity and CFOS and IL1B transcript stability were in the expected ranges for the 6 months real time storage.

Stability of PAXgene Blood RNA Kit component:
Objective: Determine the shelf life of the PAXgene Blood RNA Kit component.
Evaluation of different storage conditions via:

- Real time and accelerated stability study
- Open bottle study
- Simulated transportation study (simulated extreme temperatures, -20ºC-40ºC)

Determination of functional stability done with purified HeLa cell RNA as starting material. Two reagent sets were used per tested time point (4 blank eluates (RNase-free water as starting material), and 8 RNA eluates. The following parameters were addressed:

- Determination of recovery of input RNA (HeLa cell RNA)
- Determination of variability of recovery of input RNA
- Determination of the degree of inhibition of an RT-PCR assay (via ACTB RT-PCR monoplex assay.

Evaluation of physical and chemical parameters:

- pH
- conductivity
- density

Bioburden Analysis:

- Test of buffers for total bacterial and yeast counts per ml

Results: The RNA recovery, CV of recovery, degree on inhibition, pH, conductivity, and density (for all buffers) were in the expected ranges for the 6 months real time storage. Furthermore, the Proteinase K and the DNase I activity performed to the sponsors claims for the 6 month real time storage.

The bioburden analysis did not show any bacteria, yeast of fungi growth in the same time period. The transportation simulation also revealed acceptable performance of all kit components after extreme temperature simulation.

RNA *In Situ* Stability:
To determine the stability of RNA in samples stored at different temperatures and times, the sponsor performed several RNA *In Situ* stability experiments that are summarize in Table 2.
Objective: Evaluation of the quantity, quality and integrity of whole blood intracellular RNA after storage in the PAXgene tubes.

Table 2: Experimental Design of RNA In Situ Stability at different conditions

Storage Temperature	**2 – 8 ºC**	**18, 22, 25 ºC**	**- 20 ºC**	**- 70 ºC**	**Freeze/Thaw**
# of donors	10	10	10	10	10
Eligibility WBC per ml	1.8 to 11.0 x 10^6 cells	1.9 to 11.0 x 10^6 cells	1.10 to 11.0 x 10^6 cells	1.11 to 11.0 x 10^6 cell	1.12 to 11.0 x 10^6 cells
PAXgene tubes/donor	12	14	14	10	6
Total # of tubes Used in experiment	120	140	140	100	60
Storage before start of experiment	2 hrs RT	2 hrs RT	2 hrs RT	2 hrs RT	24 hrs RT
Time points (# of tubes)	t_0 (2 tubes) t_{1D} (2 tubes) t_{2D} (2 tubes) t_{3D} (2 tubes) t_{4D} (2 tubes) t_{5D} (2 tubes)	t_0 (2 tubes) t_{1D} (2 tubes) t_{3D} (2 tubes)	t_0 (2 tubes) t_{1m} (2 tubes) t_{2m} (2 tubes) t_{3m} (2 tubes) t_{4m} (2 tubes) t_{5m} (2 tubes) t_{6m} (2 tubes)	t_0 (2 tubes) t_{3m} (2 tubes) t_{6m} (2 tubes))	t_0 (2 tubes) 1st freeze/thaw (2 tubes) 2nd freeze/thaw (2 tubes)
Yield (> 3 μg/tube)	260 nm	260 nm	260 nm	260 nm	260 nm
Purity (ratio 1.8-2.2)	260nm/280nm	260nm/280nm	260nm/280nm	260nm/280nm	260nm/280nm
CFOS/18S rRNA	X	X	X	X	NA
Il1b/18S rRNA	X	X	X	X	X

The RNA yield, purity, integrity based on the sponsors developed Q-RT-PCR assays showed that all samples at all the time points, temperatures and freeze/thaw cycles were within the acceptance range for the individual parameters (one exception). The sponsor limited the samples to blood donors with White Blood Cell (WBC) counts between 4.8 – 11.0 x 10^6 cell/ml of blood. The performance is only evaluated for this range of WBC counts, and is specified in the package insert.

d. Detection limit:
Performance evaluation demonstrated that the PAXgene™ Blood RNA system could purify at least 3 μg of RNA with an A260/A280 ratio between 1.8 and 2.2.

e. Analytical specificity:
See above

f. Assay cut-off:
Not applicable.

2. Comparison studies:
   a. Method comparison with predicate device:
   Not applicable.
   b. Matrix comparison:
   Not applicable.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable.
   b. Clinical specificity:
   Not applicable.
   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.

4. Clinical cut-off:
   Not applicable

5. Expected values/Reference range:
   Not applicable

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.4060 with special controls. The special control guidance document "RNA Preanalytical Systems (RNA Collection, Stabilization and Purification Systems for RT-PCR used in Molecular Diagnostic Testing)" will be available shortly.