

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

Table of Contents

A.	Scope	2
B.	Definitions	2
C.	Responsibilities	3
D.	Equipment and Apparatus	3
E.	Chemicals and Reagents	4
F.	Sample Requirements	5
G.	Test Procedure.....	5
H.	Related Documents	7
I.	Attachments	9

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

NOTICE
This method may involve the use of hazardous substances and/or equipment. The user must not assume that all of the safety issues associated with its use have been described. Prior to use of this method, the user is responsible for establishing appropriate safety and health practices and determining the applicability of regulatory requirements.
The employee performing this method must be trained according to the safety guidelines specific to the job task and area of assignment. The employee must use all appropriate safety equipment referenced by the facility's safety guidelines. Copies of Material Safety Data Sheets (MSDS) are available from the Altria Safety Management intranet site, facility safety department or the area supervisor.

A. Scope

1

(b) (4)

B. Definitions

1. Multivariate Calibration - Process of relating multiple responses from an analytical instrument to reference component concentrations. The resultant multivariate calibration model is applied to the analysis of spectra of unknown samples to provide an estimate of the component concentration. The multivariate calibration algorithm employed in this method is partial least squares.
2. Near Infrared (NIR) – The Near Infrared region of the electromagnetic spectrum falls in the range of 14000-4000 cm⁻¹. This region contains overtone and combination bands of fundamental molecular vibrations that are useful for quantitative analysis. See Attachment I.
3. Partial Least Squares (PLS) – Algorithm used for quantitative spectroscopic calibration. It is based on calculating empirical factors in such a way that they can be used to estimate both the spectral data and the constituent composition in a near-optimal manner.
4. QC Check Standard – Standard solutions that are prepared with known concentration mixes that are representative of the type of samples to be tested. They are used to check the instrument's accuracy of all components prior to analysis. Upper and lower control limits are based on 3*SEP for each component for the particular model in use. The check standards are also used to assure that the chemometric model is valid.

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

5. SQC (Statistical Quality Control) – Instrument test sequences used specifically with Bomem Spectrometers to monitor correct operation of the analyzer.
6. Standard Error of Prediction (SEP) - Summary statistic used to evaluate model performance for each analyte. It is obtained using the leave-one-out cross-validation protocol, in which each calibration sample in turn is predicted from a model that has no information about that sample. The errors of prediction for all samples comprising the data set are collected to give a final estimate of the overall accuracy of the model.

C. Responsibilities

1. Laboratory management shall ensure that personnel performing this method have demonstrated the ability to properly perform this method.
2. Laboratory personnel are responsible for performing testing and documenting information as defined in this method. Any significant deviations from this method are to be documented and reported to laboratory management.

D. Equipment and Apparatus

1. Equipment and Apparatus Required
 - a. ABB Bomem MB160 or FTLA 2000 series FT-NIR Analyzer of scanning from 11000-4000 cm⁻¹
 - b. NIR Operating Software
 - c. 5mm x 42mm optical grade glass vials, GX-33 glass required, Lab Supply Distributors #20870-542
 - d. Temperature Controlled Vial Holder (TCVH) liquid sampling accessory
 - e. Lamp, 12V, 20 W, Thermo-Fisher #L9404
 - f. General Supplies
 - 1) Vial Plugs for 5mm vials
 - 2) Delicate Task Wipes (Ex.: Kimwipes®)
 - 3) 0.3 O.D. Neutral Density Filter
 - 4) Powder-free Latex or Nitrile Gloves
 - 5) Transfer Pipettes with Bulb or 1mL Pipettor with Tips
2. Instrument Setup
 - a. Refer to manufacturer's documentation for specific requirements of equipment.
 - b. Wear clean powder free latex or nitrile gloves. Fingerprints will damage equipment.
 - c. Always leave the NIR instrument and NIR source on. After a power outage, the NIR must be allowed to warm up for 2 hours. The NIR should be allowed to warm up overnight after changing the source bulb.
 - d. Ensure that the NIR is set up properly. Refer to diagrams in Attachment IV.

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

- e. Remove the red plastic window covers prior to use.
 - f. Settings listed below are for instrumentation at USSTP LPF. Settings for instruments across all facilities can be verified via the daily QC checks.
 - 1) Verify the gain settings. The gain dial must be set to High A.
 - 2) Verify the resolution dial is set to 16 cm⁻¹.
 - g. Ensure that the temperature controlled vial holder (TCVH) sampling accessory is properly installed and set to 31°C.
 - h. The TCVH must be allowed to warm up at least 5 minutes if it has been turned off and it must read 31°C prior to analysis of any samples or backgrounds. All samples, background, and standards must be placed in a TCVH at 31°C for a minimum of 5 minutes prior to analysis. An additional TCVH is available for warming samples.
 - i. Obtain a fresh plant water sample. Transfer the sample to a vial and plug the vial. Label the plug as plant water. Wipe the outside surface of the vial with a clean, lint free cloth (i.e. KimWipes®). Place the sample in the TCVH so that it is warmed and ready for analysis. Samples must be warmed a minimum of 5 minutes prior to analysis. A plant water background sample must be measured once per hour and prior to the analysis of any (b) (4) batch samples.
 - j. Obtain a fresh deionized water sample. Transfer the sample to a vial and plug the vial. Label the plug as deionized water. Wipe the outside surface of the vial with a Kimwipe®. Place the sample in the TCVH so that it is warmed and ready for analysis. Samples must be pre-warmed a minimum of 5 minutes prior to analysis. Deionized water backgrounds are measure prior to the analysis of QC Check Standards.
3. Instrument Maintenance
 - a. See instrument manual.
- E. Chemicals and Reagents
1. Chemicals Required
 - a. Calibration and Quality Control Check Standards
 - 1) Deionized Water

(b) (4)

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

9) (b) (4)

NOTE: Materials are obtained from plant stock materials and should be representative of materials to be analyzed.

2. Reagent Preparation

a. N/A

3. Standard Preparation

a. Calibration samples are to be prepared using a laboratory scale with reference values given in weight percent.

b. Atomic weights are used to determine portion of given salt that is contributed by given ion.

c. (b) (4)
Reference values and upper and lower specification limits are provided with the QC Standards. The specification limits are set at 3*SEP for each component analyzed.

d. The (b) (4) Check Standards are generally prepared by UST personnel in Nashville and distributed to other UST and Altria laboratories that analyze (b) (4). (b) (4) Check Standards are stored in a refrigerator with the exception of (b) (4) which is stored at room temperature due to issues with precipitation.

e. For the week the standards are in use, they are not refrigerated and must be discarded after one week. Standards have an expiration date and should not be used past the expiration date.

F. Sample Requirements

1. Approximately 250µl of sample is needed to fill sample vial, although approximately 200ml is sampled from process tanks.

G. Test Procedure

1. Sample Handling

a. N/A

2. Calibration

a. This method is based on multivariate calibration models and development of these models is outside of the scope of this method.

3. Analysis

a. After analysis of QC Check Standards you may analyze Batch Samples. Click "Sample Analysis". Click "Ok". Click the name of the product you are testing (b) (4) for. Click "Ok". You will be prompted to place a Plant Water reference in the TCVH. Click "Ok". Click on the product you are analyzing. Enter the Sample Code and Analyst initials. Click "Ok". Click "Print" and "Ok".

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

- b. Refer to the Tables listed under Attachment II and draw a single line through any values that are Below the Limit of Quantitation (BLOQ) for that component and write BLOQ next to the value. Components with values Below the Limit of Quantitation (BLOQ) will be reported as BLOQ for that component. Numerical values will not be reported for any components that are BLOQ.
 - c. Retain the printout in a binder. This will take you back to the product screen where you may select a product and continue to analyze additional samples. Click "Cancel" when all analyses are completed. This will take you to the main menu. Click "Exit Ahrs" at the end of the working day.
 4. Calculations and Reporting
 - a. Calculations are based on calibration predictions and are embedded in the model and are beyond the scope of this test method.
 5. Quality Control and Acceptance Criteria
 - a. System Suitability
 - 1) Instrument Performance Qualification Checks are required and must pass prior to any analysis. These tests include: energy level, photometric noise, photometric precision and linearity, and frequency validation. Basic tests expire daily and extended tests expire weekly. See Attachment III for a typical Bomem instrument performance qualification check.
 - b. Login to the NIR computer. You will be prompted to verify computer date and time is correct. If incorrect, follow prompts on screen to correct. Click "Ok".
 - c. The "Instrument Test Expired Screen" will appear if any instrument tests have expired. The Basic Test expires once every 24 hours and the Extended Test expires weekly.
 - d. Click on "SQC", "Basic", "Liquid Sampler" to run the Basic Instrument test. You will be prompted to insert a Plant Water background into the center hole of the TCVH. Type Plant Water and enter the date collected and select Ok. You will be prompted to set resolution to 16 cm⁻¹ prior to analysis. Type "Plant Water" and the date the water was collected in the date field and click "Ok".
 - e. Ensure the Basic instrument test passes. If the test passes, the screen will be green and the Basic Test Result Screen will read "Passed 1 Spectral Quality Test of 1". Click "Ok" and proceed. If the test fails, the screen will be red and the Basic Test Result Screen will read "Passed 0 Spectral Quality Tests out of 1". Samples may not be analyzed until the test passes. Click "Ok", "Ok", "Print Report and Exit", "Ok" to exit the Basic Testing screen.
 - f. If prompted, run the Extended Instrument Test.
 - g. Click "Extended", and "Liquid Sampler", "Ok".
 - h. Place the pre-warmed vial of plant water in the center hole of the TCVH. You will be prompted to set the resolution to 2 cm⁻¹. Click "Ok". Enter "Plant Water", date, and your initials. Click "Ok". You will be prompted to set Gain to

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

Low A. For instrumentation at USSTP LPF, the gain should be set to High A for this instrument.

- i. If the test passes, the screen will be green and the Extended Test Result Screen will read "Passed 1 Frequency Validation Test of 1". Click "Ok" and proceed. If the test fails, the screen will be red and the Extended Test Result Screen will read "Passed 0 Frequency Validation Tests out of 1". Samples may not be analyzed until the test passes. Click "Ok", "Exit", "Ok", "Exit" to exit the Extended Testing screen.
- j. Set resolution at 16 cm-1. Click "Ok". Another basic test will be performed. If the test is passed, the screen will be green and the screen will read "Passed 1 Spectral Quality Test of 1". Click "Ok" and proceed. If the test fails, the screen will be red and the Basic Test Result Screen will read "Passed 0 Spectral Quality Tests out of 1". Samples may not be analyzed until the test passes. Click "Print Report and Exit", "Ok" to exit the Basic Testing screen. Click "Cancel" to return to main menu.
- k. After instrument tests have passed, proceed to analysis of (b) QC Check Standards that are relevant to expected production batch samples (b) (4)
[REDACTED]
- l. Refrigerated Check Standards must be warmed to room temperature for a minimum of 20 minutes and vigorously shaken to dissolve all salts prior to vialing. Vial each of the QC Check Standards and cap. Label each cap distinctly. Place vials in TCVH to pre-warm for 5 minutes prior to analysis. Click on "Sample Analysis" "Ok", (b) QC Standards, "Ok". You will be prompted to analyze a deionized water background prior to analysis of QC Standards. Place deionized vial in TCVH and click "Ok".
- m. Proceed to analyze the Check Standards. You will be prompted to place the QC Standard in the TCVH. Click "Ok". Click the appropriate type of (b) (4) (b) (4) for data analysis. Enter the name of the Standard and your initials prior to analysis. Click "Ok". Print results and verify that each component meets the established specification for that component. Click "Ok". Retain the printouts in a binder.
- n. If the QC Check Standards do not meet specifications, reanalyze. If the QC Check Standards are still outside of specifications after reanalysis, consult with your supervisor prior to analysis of any batch samples. See Attachment V for troubleshooting direction.
- o. Click "Cancel" to exit analysis of QC Check Standards.

H. Related Documents

1. Validation Report Rev 01. 2011. Determination of (b) (4) Components by Near Infrared.

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

2. Validation Plan Rev 01. 2010. Determination of (b) (4) Components by Near Infrared, November 16, 2010.
3. Supplemental Validation Rev 01.1. Determination of (b) (4) Components by Near IR.
4. MB Series Spectrometers Reference Manual, Revision 1.0, April 2000.
5. Airs QA and Airs Professional Users Guide, Revision 2.3, March 2003.

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

I. Attachments

Attachment I

(b) (4)

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

Attachment II

Typical Second (b) (4) QC Check Standards Pass/Fail Criteria

	Standard ID: 017001.00 (Tosse)			Standard ID: 018001.00 (Tosse)			Standard ID: 018101.00 (crack) EP			Standard ID: 018101.00 (crack) EP		
Location Code	(b) (4)											
Component	Target	LSL	USL	Target	LSL	USL	Target	LSL	USL	Target	LSL	USL
	(b) (4)											

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

Attachment III

Typical Bomem Instrument Performance Qualification Check

INSTRUMENT TEST REPORT SUMMARY

AIRS Professional Version 2.2

Time/Date 10:27 AM 6/7/2010

Test Sequence EXTENDED

Number of Repetitions: 1

Frequency Validation Passed 1 of 1

Spectral Quality Passed 1 of 1

)))))) CONFIGURATION 3

Analyzer Description, Configuration 3

Configuration Name Liquid Sampler

Model MB160D

Serial Number SZM480RH

Sampling Accessory 5mm Vial

Sampling Device Serial Number STG1200G

Detector InGaAs

Detector Serial Number SNH4300T

####7

PASSED FREQUENCY VALIDATION TEST

Repetition 1 of 1

Comment:

Spectrum Name: A1Z36468

Peak Number: 1

Location in Spectrum: 7299.951

Target Frequency: 7300.000

Pass Range: 7300.100 to 7299.870

Baseline Start: 7296.799

Baseline End: 7296.799

Peak Height: 7.458303

Minimum Height: 1.000000

^

####3

PASSED SPECTRAL QUALITY TEST

Repetition 1 of 1

Single Beam Test Spectrum A1Z36469.RRF

% Transmittance Test Spectrum A1Z36470.RSM

Comment

Maximum Energy 33.01253 Arbitrary units

Non-linearity 0.0055386 Percent

Modulation Efficiency 98.31352 Percent

Purge Level 100.0000 Percent

RMS Noise at 6000.000 0.0008549 Percent

RMS Noise at 7850.000 0.0006597 Percent

Mean Value at 6000.000 100.0276 Percent

Mean Value at 7850.000 100.0358 Percent

^

Non-Linearity Pass 0.1000000 %

Modulation Efficiency Pass 18.00000 %

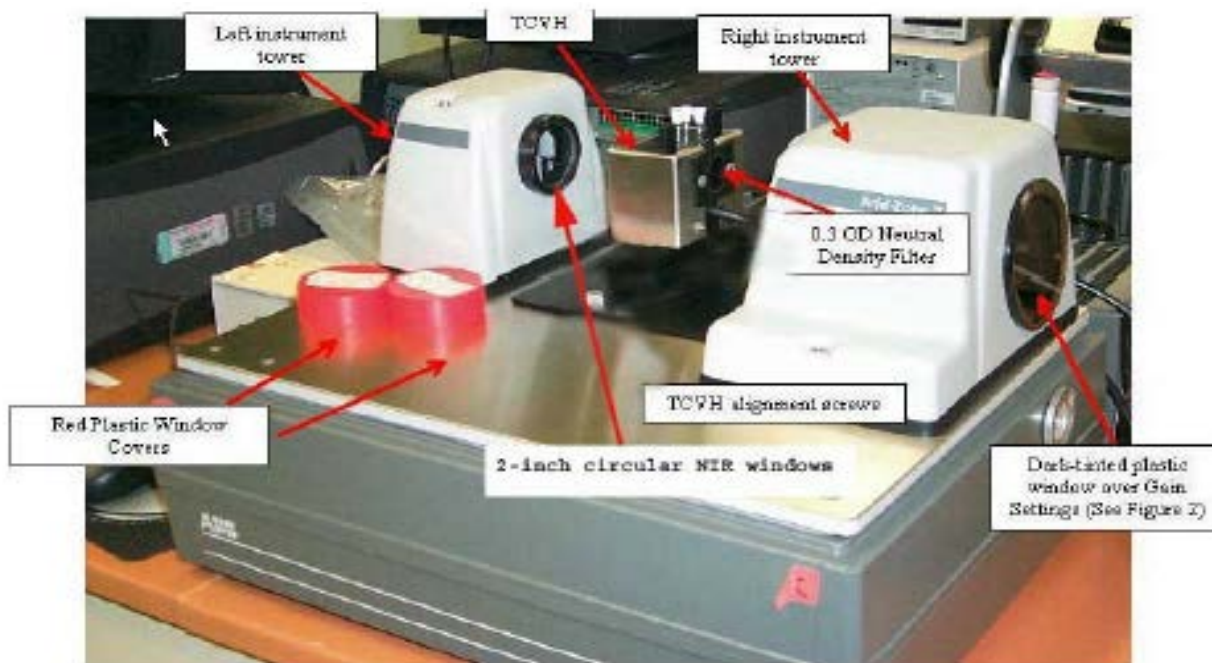
Purge Pass 1%

Baseline Deviation/Tilt Pass 2.000000 %

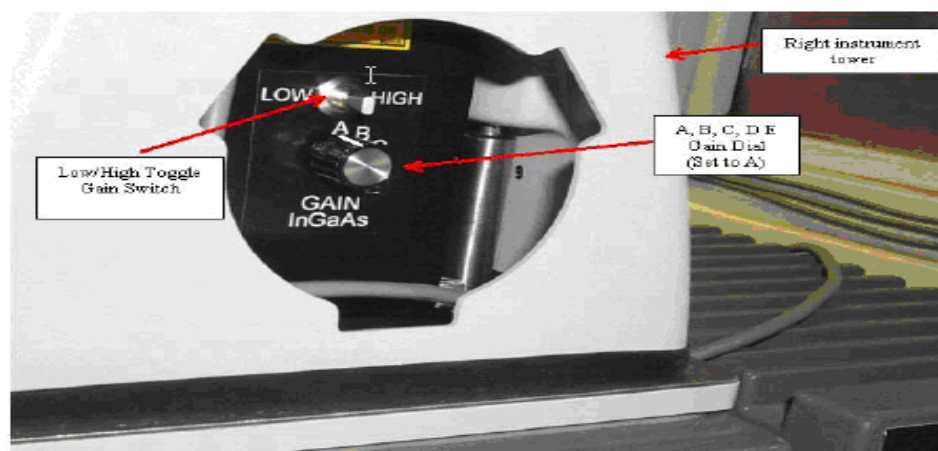
RMS Noise Pass 0.0049999 %

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

Attachment IV
Near IR Analyzer



Near-Infrared (NIR) Analyzer with Temperature Controlled Vial Holder (TCVH) Accessory

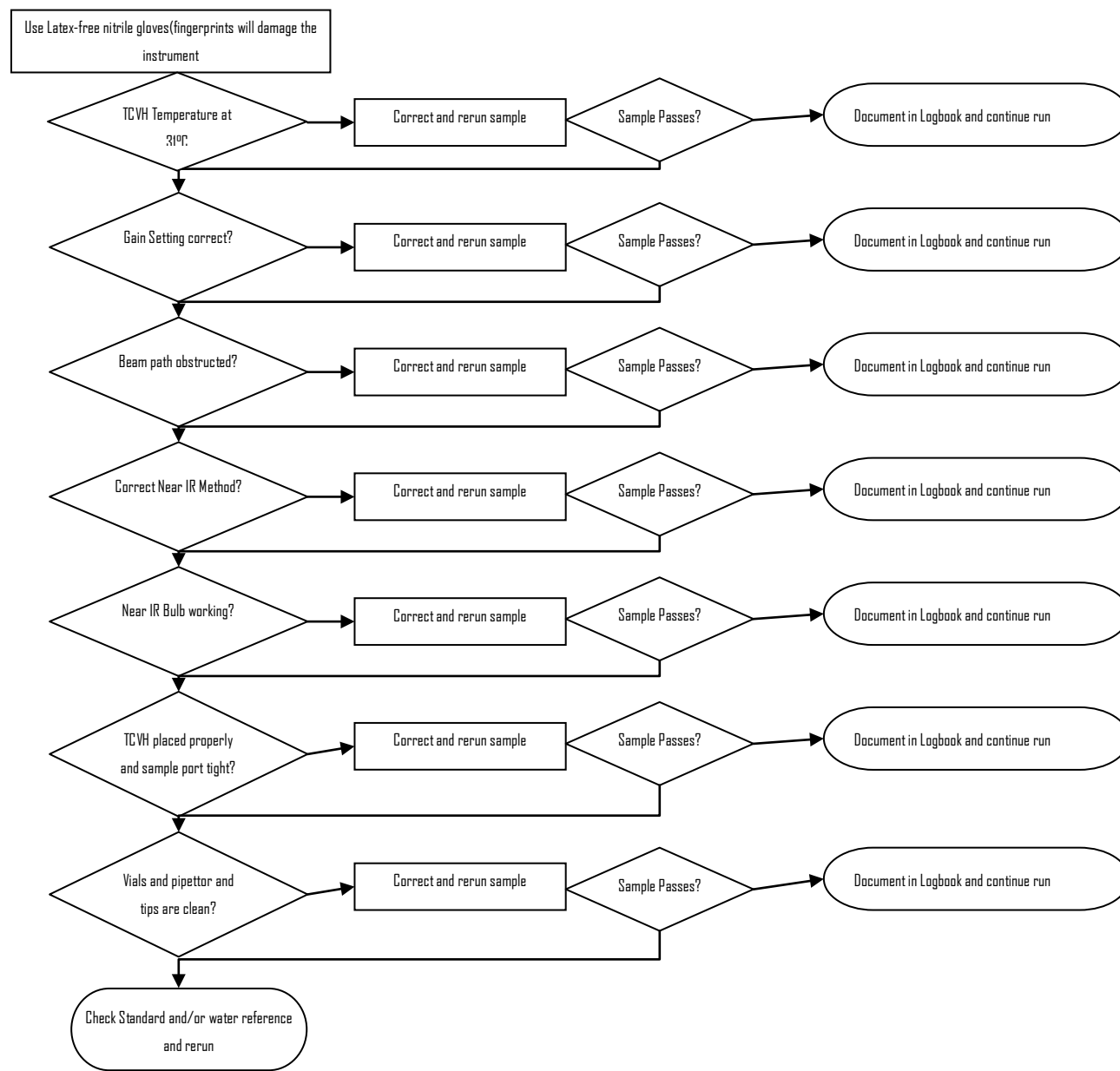


Left side of the Near-Infrared (NIR) Analyzer Gain and Resolution Settings (Dark-Tinted Plastic Window Removed)

Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

Attachment V Troubleshooting Guide

The following steps are recommended techniques to be used to determine if a consecutive out of specification check sample is instrument or reagent related. Not all steps are required, however the order the troubleshooting is to be performed is listed. Any troubleshooting performed must be documented in the instrument logbook along with routine and preventive maintenance.



Title: Determination of (b) (4) Components by NIR	Control #: ST-TM-235-104	Revision #: 1.2
	Approval Date: 12/16/2013	Effective Date: 12/16/2013
Test Method Owner: J.A. Sampson, ALCS RD&E Analytical Technical Services		

Attachment VI

(b) (4) Methods

The following models and ranges have been validated for use in this method. Products to be measured must fall within the calculated concentrations listed in order to be relevant to the model chosen. Analytes with values below LOQ will be reported as BLOQ.

(b) (4)

Component Statistics	(b) (4)
Min	
Max	
LOQ	

(b) (4)

Component Statistics	(b) (4)
Min	
Max	
LOQ	

(b) (4)

Component Statistics	(b) (4)
Model Min	
Model Max	
LOQ	