Operation Manual

PhD™ System, 110 V

REF 426-0000

PhD™ System, 220 V

REF 426-0000EX

PhD™ Workstation with Accessory Pack

REF 426-0218

PhD™ System Components (for N. America)

REF 426-0244

PhD™ System Components (for export)

REF 426-0244EX

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BIO-RAD

PhD™ System

CE
IVD

Mfd. For
EC REP

Bio-Rad Laboratories, Inc., Hercules, CA 94547, USA
by Bio-Rad Strasbourg, Schiltigheim, France
***FRANCE,** Bio-Rad, Marnes-la-Coquette*

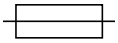
Multiple Language CD

This manual includes a multiple language CD-ROM in the following languages:
English, German, French, Spanish, Italian, Portuguese, Swedish, Danish, Greek, and Czech.

In Vitro Diagnostic Directive (IVDD, 98/79/EC) Symbols

CE • European Conformity • EG-Konformität • Conformité européenne • Conforme a la normativa europea • Conformità Europea • Conformidade com as normas europeias • Uppfyller EU-direktiv • CE-mærkning • Συμμόρφωση με τα ευρωπαϊκά πρότυπα • Evropská shoda	EC REP • Authorized Representative in the European Union • Autorisierter Vertreter in der Europäischen Union • Représentant agréé pour l'Union Européenne • Representante Autorizado en la Unión Europea • Rappresentante autorizzato per l'Unione Europea • Representante Autorizado da União Europeia • Auktoriserad EU-representant • Autoriseret repræsentant i EU • Εξουσιοδοτημένος αντιπρόσωπος στην Ευρωπαϊκή Ένωση • Autorizovaný zástupce v Evropské unii
IVD • For In Vitro Diagnostic Use • In-vitro-Diagnostikum • Utilisation comme test de diagnostic in vitro • Para uso en diagnóstico in vitro • Per uso diagnostico in vitro • Para uso em diagnóstico in vitro • För in vitro-diagnostiskt bruk • Til in vitro-diagnostisk brug • Για in vitro διαγνωστική χρήση • Pro diagnostické použití in-vitro	 • Consult Instructions for Use • Gebrauchsanleitung beachten • Consulter la notice d'utilisation • Consulte las instrucciones de uso • Fare riferimento alle Istruzioni per l'uso • Consulte as instruções de utilização • Se bruksanvisning före användande • Se brugsvejledningen • Συμβουλευθείτε τις οδηγίες χρήσης • Viz pokyny pro použití
 • Manufacturer • Hersteller • Fabricant • Fabricante • Produttore • Fabricante • Tillverkare • Producent • Κατασκευαστής • Výrobce	REF • Catalog Number • Katalognummer • Référence • Número de catálogo • Numero di catalogo • Número de catálogo • Katalognummer • Katalognummer • Αριθμός καταλόγου • Katalogové číslo
SN • Serial Number • Seriennummer • Numéro de série • Número de serie • Numero di serie • Número de série • Serienummer • Serienummer • Αριθμός σειράς • Sériové číslo	Mfd. For • Manufactured For • Hergestellt für • Fabriqué pour • Fabricado para • Prodotto per • Fabricado para • Tillverkas för • Produceret for • Κατασκευάστηκε για • Vyrobeno pro
 • Temperature Limit • Temperaturgrenze • Limite de température • Límite de temperatura • Limite di temperatura • Limite de temperatura • Temperaturgränser • Temperaturområde • Όριο θερμοκρασίας • Teplotní mez	

Instrument Symbols

 • Caution • Vorsicht • Attention • Precaución • Attenzione • Cuidado • Försiktighet • Forsigtig • Προσοχή • Upozornění	 • Fuse • Sicherung • Fusible • Fusible • Fusibile • Fusível • Säkring • Sikring • Ηλεκτρική ασφάλεια • Pojistka
 • Protective Conductor Terminal • Schutzleiter-Anschlusspunkt • Borne du conducteur de terre • Borne del conductor de tierra • Terminale del conduttore protettivo • Terminal do condutor protector • Jordklämma • Beskyttelseslederklemme • Ακροδέκτης προστατευτικού αγωγού • Ochranná zemnicí svorka	 • Moving Parts • Sich bewegende Teile • Pièces mobiles • Piezas móviles • Parti in movimento • Peças em movimento • Rörliga delar • Bevægelige dele • Κινούμενα μέρη • Pohyblivé součásti
 • Waste from Electrical and Electronic Equipment • Abfall von elektrischen und elektronischen Geräten • Déchets d'équipements électriques et électroniques • Residuos de Aparatos Eléctricos y Electrónicos • Rifiuti da attrezzature elettriche ed elettroniche • Resíduos de Equipamento Eléctrico e Electrónico • Avfall från elektrisk och elektronisk utrustning • Affald fra elektrisk og elektronisk udstyr • Απόβλητα ηλεκτρικού και ηλεκτρονικού εξοπλισμού • Odpad tvořený elektrickými a elektronickými přístroji	 • Sharp Biohazard • Infektionsgefahr durch spitze Gegenstände • Objets piquants/tranchants posant un risque biologique • Peligro biológico de objetos cortantes • Rischio biologico elevato • Perigo biológico por instrumentos afiados • Biologisk risk med vasst föremål • Alvorlig biologisk fare • Βιολογικός κίνδυνος από αιχμηρά αντικείμενα • Biologické nebezpečí - ostré předměty
 • Biohazard • Infektionsgefahr • Risque biologique • Peligro biológico • Rischio biologico • Risco biológico • Biologiskt riskavfall • Biologisk risikomateriale • Βιολογικός κίνδυνος • Biologické nebezpečí	 • Laser Radiation • Laserstrahlung • Rayonnement laser • Radiación láser • Radiazione laser • Radiação Laser • Laserstrålning • Laserudstråling • Ακτινοβολία λέιζερ • Laserové paprsky

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TECHNICAL ASSISTANCE

Bio-Rad provides a toll-free line for technical assistance, available 24 hours a day, 7 days a week.

In the United States of America and Puerto Rico call toll-free 1-800-2BIORAD (224-6723).

Outside the U.S.A., please contact your regional Bio-Rad office for assistance

Warning *Caution*

Do not remove the instrument case.

There are no user-serviceable parts inside.

Refer servicing to Bio-Rad personnel.

The PhD™ System satisfies the safety requirements defined in the In Vitro Diagnostic Medical Devices Directive, 98/79/EC. Certified products are safe to use when operated in accordance with the instruction manual. This certification does not extend to other equipment or accessories not certified, even when connected to the PhD System.

This instrument should not be modified or altered in any way. Alteration of this instrument will void the warranty, void the certification, and create a potential safety hazard for the user.

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

Biohazard

Please read through and familiarize yourself with the contents of the operation manual before using the system for the first time.

All personnel using the PhD System should wear standard laboratory safety apparatus (e.g., safety glasses, laboratory coat, gloves).

All reference material (i.e., calibrators, controls) and patient samples should be considered as biohazardous material and must be handled with caution.

Waste material contains sample waste plus reagents; treat and dispose of as directed by your laboratory safety guidelines or by local ordinance.

Sharp Biohazard

Use caution when handling the probe tip to avoid injury. The used probe tip should be considered potentially biohazardous; discard according to the laboratory standard operating procedures for biohazardous sharps.

Chemical Hazards

Test kit components may contain potentially harmful chemical materials. Follow all instructions for handling, storage, and disposal as described in the appropriate method instruction manual.

General Precautions

- Consult the Material Safety Data Sheets (MSDS) for specific safety information.
- Do not smoke, eat, or drink in areas where reagents are handled.
- Wear personal protective equipment while handling all reagents.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.

FCC (Federal Communications Commission) Compliance

This equipment complies with the requirements in part 15 of FCC Rules for Class A computing device. Operation of this equipment in a residential area may cause unacceptable interference to radio and TV reception requiring the operator to take whatever steps are necessary to correct the interference.

Electrical Hazards

Do not remove the instrument case. There are no user-serviceable parts inside. Refer all servicing to Bio-Rad service personnel.

NOTE: *The main power cord on the right side of the instrument serves as the primary power disconnect. Do not position the PhD System in a position where it is difficult to disconnect the main power cord.*

Electrical and Electronic Waste Hazards

The Waste Electrical and Electronic Equipment (WEEE) Regulations implement provisions of the European Parliament and Council Directive 2002/96/EC aimed at reducing the amount of EEE waste going for final disposal. As the producer, Bio-Rad Laboratories, Inc. has specific instructions for the recovery of this instrument at the time of end of use. Please go to www.bio-rad.com for the process applicable to your region.

Laser Radiation Hazards

Please review the barcode scanner User's Guide, included in the PhD System accessories box, for important safety information.

Definition of Symbols

NOTE: Note statements alert you to important information that is relevant to the current subject matter.

Caution: This icon alerts you to a condition that may cause damage to equipment or data.

Warning: This icon directs you to follow specified instructions where safety is involved.

Biohazard: This icon alerts you to a potentially biohazardous condition.

Sharp Biohazard: This icon alerts you to a potentially biohazardous sharp condition.

Trademarks and References

PhD is a trademark of Bio-Rad Laboratories, Inc.

StatLIA is a registered trademark of Brendan Technologies, Inc.

NOTE: The curve fitting functions were developed by Brendan Technologies, Inc., and are used in its StatLIA software.

All other trademarks are the property of their respective companies.

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1 Introduction

The Bio-Rad PhD™ System is an EIA and IFA processing system that consists of a PhD fluid handling station(s) linked to a network computer via an ethernet hub. The network computer provides worklist generation, data management, data reduction and microplate reader control functions. As testing volume increases, up to ten PhD Workstations can be added to a single network computer. The system interfaces with a microplate reader and to a Laboratory Information System. The network computer operates in a Microsoft® Windows® 2000 environment using icon-based software commands. This modular system is a high-throughput analyzer capable of handling up to 8 assays with up to 192 tests (including calibrators and controls) for EIA, or 4 assays with up to 96 tests for IFA, in a batch on each workstation. Refer to the PhD IFA Module Operation Manual for performing IFA testing on the PhD System. The PhD System is shown in Figure 1-1.

The PhD System includes the following components (the PhD printer is not shown):

① PhD Workstation
② Computer Monitor
③ Microplate Reader
④ PhD Numeric Keypad
⑤ Computer Keyboard
⑥ PhD Computer
⑦ Barcode Scanner
⑧ Mouse

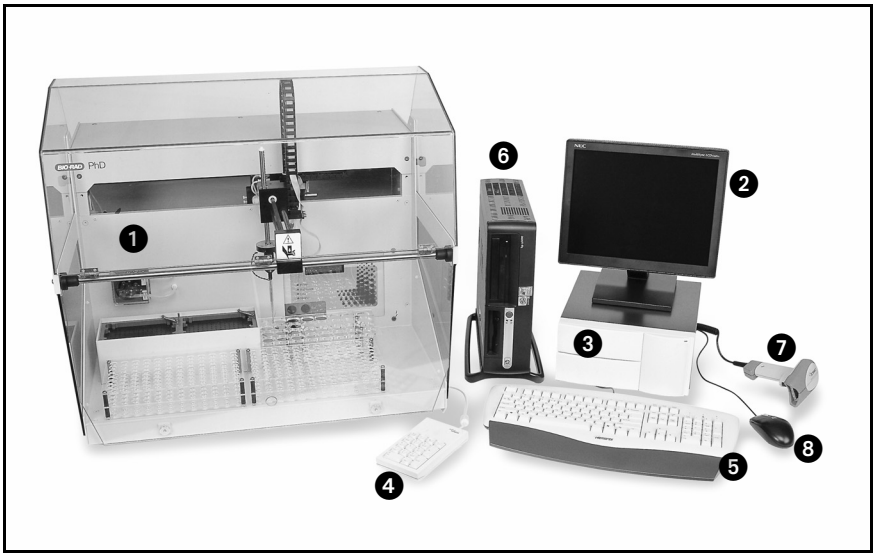

Figure 1-1 The PhD System

1.1 Principles of Operation

The proprietary volumetric pump allows the workstation to accurately and consistently deliver volumes as low as one microliter. This eliminates the need for predilution of patient samples, allowing direct dilution in the microplate wells. After dilution of samples, the system incubates, washes and adds reagents to the microplate sequentially. Incubations for each well are timed individually, thus ensuring uniform timing across each plate.

A polycarbonate cover encloses the moving parts and samples during processing, yet allows for observation of the operations. A barcode scanner allows entry of sample and plate IDs.

The software is flexible, intuitive, and easy to learn for simple programming of assay runs. It is also open to programming new assays from multiple manufacturers. Along with extensive data reduction capabilities, the software communicates results to a Laboratory Information System and generates hard-copy reports.

NOTE: *Any new assays added to the system must be validated according to the laboratory's standard operating procedures before reporting results.*

2 System Description

2.1 Introduction

The PhD System includes the PhD Workstation, computer, printer, microplate reader and barcode scanner.

2.2 System Components

The PhD Workstation is a liquid handling robotic unit developed for use with standard 96-well microplates. The instrument is designed to move samples, reagents and wash around the workstation using a dual probe tip in conjunction with the volumetric and peristaltic pumps.

The PhD is capable of processing two 96-well microplates (192 individual wells) simultaneously. It has two removable sample racks for 192 sample tubes, 12 mm x 75 mm or 13 mm x 100 mm in size. Additional sample rack sizes are available (see Appendix A). An internal warm air incubator, under the microplate platform, allows assays to be run at temperatures from 25–40 °C. The fixed reagent rack holds up to 31 reagent bottles.

The fluid handling functions on the workstation deck are performed using the volumetric pump, peristaltic pump and probe tip. The probe tip consists of two tubes. The short tube is used for the aspiration and dispensing of samples and reagents in addition to the dispensing of wash solution from an external wash bottle; this function is accomplished by the volumetric pump. The longer tube aspirates waste, through the action of the peristaltic pump, to the external waste bottle.

The PhD computer (minimum specification) consists of an Intel® Celeron® processor, 20 GB hard drive, 128 MB RAM, USB and serial port connectivity. The PhD uses a color inkjet printer and an easy-to-use PC-driven microplate reader. The barcode scanner is an input device that can read a barcode from a distance of 6 to 20 inches.

Figure 2-1 shows some of the external workstation components.

Figure 2-1 The PhD Workstation

① Sample Racks (2 x 96)
② Reagent Rack
③ Microplate Platform
④ Peristaltic Pump
⑤ Probe Tip
⑥ Probe Holder
⑦ Clear Cover

2.3 Liquid Handling

The Aspiration/Dispense Tube is connected, through a combination of silicone and Teflon® tubing, to the volumetric pump. It is used for the aspiration and dispensing of samples and reagents on the workstation deck. It also handles the dispensing of wash solution, aspirated by the volumetric pump, from either of the external wash bottles. Wash selection is regulated by an electromagnetic pinch valve.

The Waste Tube is connected, through a combination of silicone and Teflon tubing, to the Peristaltic Pump. The waste is evacuated from either the microplate wells during the wash step, or from the wash cup during probe tip washing. The waste is deposited into the external waste bottle.

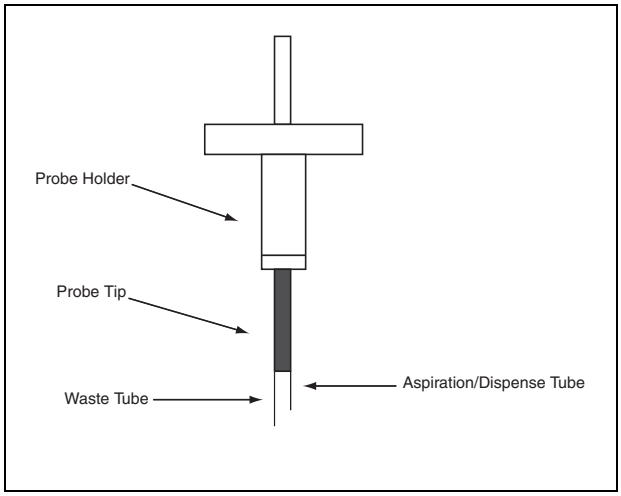

Figure 2-2 Probe Holder and Tip

2.4 Specifications

2.4.1 PhD Workstation

Operating Environment

- Ambient temperature: 15 to 27 °C
- Humidity 10–90%, non-condensing
- Safety: Class 2
- Pollution: Class 2
- Altitude: 2000 m maximum

Power Requirements

- Volts: 100 to 240
- Hz: 50 to 60
- Watts: 65 to 200 W maximum

Fuse

- 5 x 20 mm, M 6.3 A / 250 V
- UL listed fuse only

CAUTION: For continued protection against risk of fire, replace only with the same type and rating of fuse.

- Overvoltage category: II

Dimensions of PhD Workstation

- Depth: 560 mm (22 inches)
- Width: 710 mm (28 inches)
- Height: 710 mm (28 inches)

Weight

- 35 kg (77 lb) - uncrated
- 80 kg (176 lb) - crated

Fluidics

- Self contained fluidics (modular design)
- One volumetric pump with valveless ceramic piston
- One peristaltic pump (for waste)

Sampling Station

- Sample rack: Two 96-well racks
- Sample tubes: Accepts 12 x 75 mm or 13 x 100 mm sample tubes; additional rack sizes available; dead volume 200 µL for round-bottom tubes
- Probe mechanisms: Closed loop servo-motor feedback system
- Reagent rack: One 32-well rack accepts 31 (28 x 80 mm) bottles; dead volume 1.5 mL
- Incubator range: 25 to 40 °C
- X, Y, Z pipetting arm

Wash Bottles

- Two 1-L PETG bottles with caps

Waste Bottle

- One 2-L polyethylene bottle

Interface Ports

- 1-RS232 serial port (for internal use only)
- 1-Ethernet network
- DIN keypad port

Pipetting Control Computer

- Controls robotic functions - align, prime, select and run worklists
- Flash card retains instrument alignment and temporarily stores worklists

Numeric Keypad

- Controls local functions - priming, alignment, precision check and worklist selection.

2.4.2 Personal Computer Specifications (minimum)

- Microprocessor: Intel 333 MHz Celeron processor
  Memory: 128 MB RAM
- Hard drive: 20 GB
- Ports: 4-USB, 2 9-pin com ports
- Keyboard: Standard PS/2
- Mouse: PS/2
- Monitor: 15″ flat panel LCD
- Operating System: Microsoft Windows 2000 Professional
- CD-ROM Drive

2.4.3 Peripherals

Barcode Scanner

- PSC® QuickScan® 6000+ or equivalent
- Barcode scanner cable

UPS

- APC® BackUPS® 650 or equivalent

Printer

- HP® DeskJet® 3940 (with ABL USB 6 ft. printer cable) or equivalent inkjet printer

Interface Ports

- 4-port ethernet hub or equivalent - for connecting 3 PhD Workstations

  NOTE: *A maximum of 10 PhD Workstations can be linked together using an optional hub.*

2.4.4 Reader

- PR3100 (Remote Control) with 405, 450, 550 and 620 nm filters or equivalent reader
- Absorbance Range: 0 to 4.00 at 400–750 nm

2.5 Precautions

The following instructions are offered to assure safety and to prolong the life of the PhD System.

NOTE: *Always operate the PhD with the protective cover closed.*

- Be sure the PhD is placed on a level, secure surface.
- Do not open the back panel while the power is on.
- Do not attempt to make any repairs or adjustments to the circuitry. Use only the service parts and accessories supplied by Bio-Rad Laboratories.
- Do not disconnect the electrical connection while the power is on.
- Do not continue to operate a malfunctioning PhD Workstation. Call Bio-Rad Technical Service immediately if a malfunction occurs.
- Read the appropriate section of this manual prior to operating the PhD System.

2.6 Workstation Network Diagram

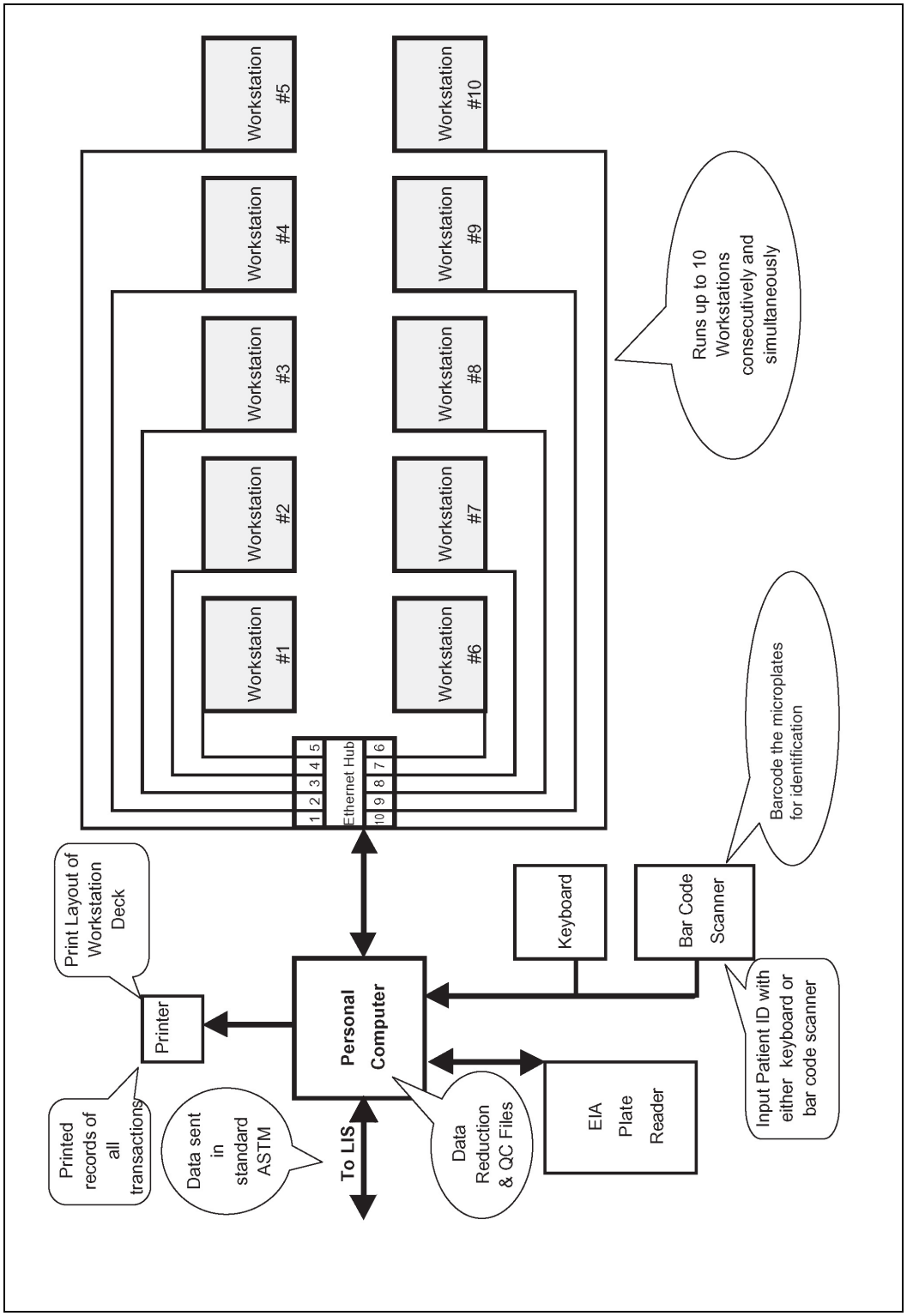

Figure 2-3 Workstation Network

3 Installation

Installation of the PhD System should be performed only by an authorized Bio-Rad representative. Installation by any other person will invalidate the system warranty.

Retain the shipping crates and packing material for use should the unit need to be repackaged and shipped.

3.1 Installation Requirements

1. Choose a location for the system that is away from direct sunlight and is relatively dust-free.

2. Room temperature should be between 15 to 27 °C.

3. The bench or table should have a flat level surface that is free from vibrations and is capable of supporting up to 94 kg (approximately 200 lb).

4. The minimum bench space required for the complete system is 89 cm deep x 168 cm wide x 89 cm high (35″ deep x 66″ wide x 35″ high).

4 Operation

4.1 General Information

- Perform all applicable Daily Startup Maintenance procedures before starting the first run of the day. See the Daily Startup checklist in Section 5.1.
- All kits and reagents should be handled according to the package insert.
- The main features of the software, provided in Section 4.2, should be reviewed first, followed by Section 4.3: Programming a Worklist for Processing.

4.2 PhD Software Overview

Each workstation connected to the PhD computer has an associated workstation directory in the PhD software. Instruments 1 through 10 correspond to the ND1 through ND10 directories. All worklists for immediate processing are saved to one of these directories as a .wel file. The file name is defined when the worklist is saved. A .wel file contains all information from the assay file(s) that is required to process the programmed worklist. ND0 is a backup directory that contains copies of the firmware files for the workstation(s) and can be used to save worklists that will be reused at a later time. Worklists stored in the ND0 directory will not be available for immediate processing.

Each ND directory, except ND0, contains a Done folder. Completed worklists are automatically transferred to this folder, from the main ND directory, when a worklist has completed processing on the corresponding workstation. Once the plate(s) is read, the OD values for each well are added to the .wel file. A report can then be generated based on the information contained in the file.

4.2.1 Main Menu Options

File

This menu contains the following options:

- **Open:** Opens a previously programmed and saved worklist (.wel file). This can be a processed or unprocessed worklist. The default directory C:\Program Files\Bio-Rad\PhD\Shared\ND1 will open. If the file was not saved in this directory, the user can navigate to the appropriate directory to open the file. If a processed worklist is opened, the worklist layout will open on the screen. To review the results of the file, select the Review Results menu option instead of Open.
- **Save as:** Saves a programmed worklist for reuse only. Saving a .wel file in this way makes it unavailable to the workstation for immediate processing. The default directory C:\Program Files\Bio-Rad\PhD\Shared\ND1 will open. If this is not the desired directory, the user can navigate to another directory to save the file. This function is useful when creating worklist templates for routine daily setup. The file can be opened when needed and have the current sample ID information programmed before saving to an instrument for processing.
- **New:** Allows a new worklist (.wel file) to be created. The new worklist can be saved for reuse (Save As) or saved (Save to Instrument) for immediate processing.
- **Print Worklist:** Prints a copy of the programmed worklist displayed on the screen. The printout contains the Worklist Name (if it was saved first), the tests ordered, and the sample ID for each sample programmed.
- **Print Screen:** Prints a copy of the PhD software screen. This function is useful if there are multiple workstations networked to the computer. The color printout can be taken to the workstation as a guide for positioning the reagents, calibrators, controls, samples and microplates.
- **Exit:** Exits the main software.

4.2.2 Save to Instrument

Allows a programmed worklist (.wel file) to be saved to the appropriate workstation directory for immediate processing. There are 10 Instrument options plus a Temporary (ND0) directory. If the Temporary option is used, the worklist will not be available for immediate processing. The user saves the worklist to the Instrument number where it will be run. The Instrument number can be found on the LCD panel of the workstation.

4.2.3 Reader Action

- **Archive:** Allows the user to place completed worklists (.wel files), from the Done directories for each workstation, into a designated backup folder.
- **Precision Check:** Reads a completed precision check plate and performs calculations. A Precision Check is required as part of quarterly maintenance.
- **Manual Read:** Allows a completed microplate to be read outside of a programmed worklist. This option will produce raw OD values, in milli OD, only. No results will be calculated.
- **Open Reader Door:** Allows the user to open the reader door to insert and read the microplate, or to remove a microplate that was read.
- **Close Reader Door:** Allows the user to close the reader door after inserting a microplate to be read, or remove a microplate that was read.
- **Set-Up:** Lists the reader options for the software and the COM ports available.

4.2.4 Read Plate

This option is selected after plate processing by the workstation. Select the Instrument number from the drop-down list, then select the worklist name. Only instruments with completed sample runs in the Done directory for that instrument will be available. Results will be calculated and a printout available.

4.2.5 Review Results

Allows the user to review completed sample runs. Only instruments with completed sample runs, in the Done directory for that instrument, will be available. Results can be reprinted.

4.2.6 LIS

This menu has 4 options:

- Worklist -> LIS: Transmit any .wel file from the selected Instrument to the LIS
- Results -> LIS: Transmit any completed .wel file, from the Done directory for that Instrument, to the LIS
- Setup: Select options LIS Send Mode, LIS Interface, and transmission setup. (See Section 4.5.1.)
- Query LIS (F12): Sends a query to the LIS to retrieve the tests ordered for each sample ID. The query will occur when prompted by the user after one or more sample IDs have been added to the worklist. (See Section 4.5.2.)

See Appendix F for a detailed description of the LIS specifications.

4.2.7 Assay

Allows for editing of current assay files or the creation of new assay files. See Appendix E for a detailed description of this function.

4.2.8 Help

The About option shows the Version, Release and Build numbers.

4.3 Programming a Worklist for Processing

1. Open the PhD software using the icon on the desktop. The main window will open. See Figure 4-1. The top of the window shows the menu options and the available assays. The bottom of the window reflects the layout of the workstation deck. A Sample ID box is also present.

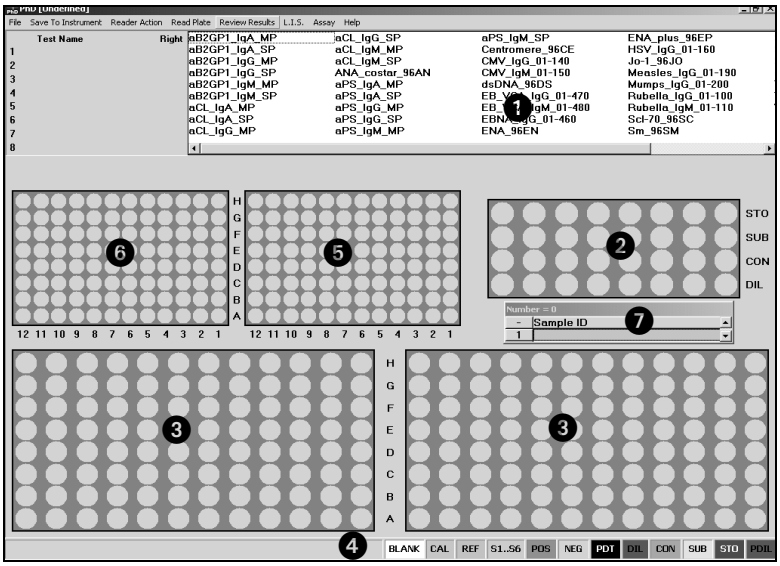

Figure 4-1 PhD Software Main Window

① List of available assays
② Reagent rack
③ Sample rack
④ Color-coded legend
⑤ Microplate - right
⑥ Microplate - left
⑦ Sample ID box

NOTE: *All tests run within the same run must have identical incubation times and temperatures. If the times are incompatible, sample results will be affected.*

2. Select the first assay to be run from the list at the top, right. Up to 8 assays can be programmed in one sample run. The color-coded reagent, calibrator, control and microwell positions will appear. The color chart appears at the bottom right of the window. Wash Bottle 1 will be selected for the wash solution. When the first assay is selected, all incompatible assays, based on the compatibility class defined in the assay file, will be eliminated from the menu. When a second assay with a different plate type

or wash solution is selected, all assays that do not share either of the programmed plate types or wash solutions will be eliminated from the menu. If an assay was selected in error, right click the assay name on the top left of the window and select "Delete". If Sample ID numbers have already been entered for this test, the numbers will remain.

3. Open the Sample ID box by clicking "-" in the upper left corner of the box. This box can be moved anywhere on the screen by clicking and holding the top bar, then dragging it to another position.

4. Enter the sample ID numbers manually or by barcode scanner.

   How to batch program the samples:

   To batch program the samples, select the assay(s) for the first sample in the batch. The selected assay(s) (e.g., 1 for assay number 1) will be shown in red. Now select the first position number (e.g., 1, 2, 3, etc.) of the batch and hold the Shift key on the keyboard as the last position number (e.g., 20, 21, 22, etc.) is selected. All samples between the first and last selection will be programmed for the same assay(s).

   How to program samples on both right and left microplates:

   All sample positions will appear in the right sample rack on the window as they are programmed, if there is an equivalent number of microwell positions. To maximize the number of programmable samples in a multi-assay run, the user can move assays to the left microplate position by unchecking the “right” option next to the test name.

   Figure 4-2 shows a programmed sample run of 2 assays with 2 full microplates and 2 different wash solutions. Note that the check mark next to the dsDNA assay has been cleared to indicate that the assay is to start on the left plate.

NOTE: *By placing the cursor over a calibrator, control, or reagent, the assay name and component information will be displayed at the bottom left of the window. For samples, the Sample ID will be displayed.*

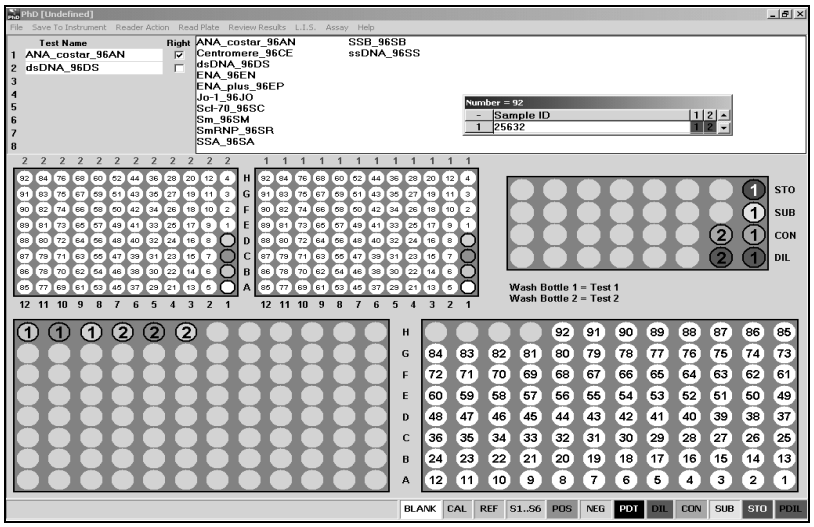

Figure 4-2 Programmed Sample Run

5. Once programming is complete, the worklist is saved to a designated workstation for processing. Select Save to Instrument from the menu at the top of the window. Select the Instrument number of the workstation to be used.

6. Enter the Operator name and Plate ID(s) if desired. Enter a Worklist Name; this is a required field and will become the name of the .wel file. Enter the Lot Number and Expiration Date for each assay.

7. For assays that require a calibration factor to calculate results, enter this value in the Scaling Factor for Result (or Cut-Off) field. This value will be used to calculate sample results for this run only. The default value in the assay file will not be affected. If a scaling factor is changed when it is not required for an assay, a message will appear warning that altering the value will affect the results. If a scaling factor is not changed for an assay requiring an update, a message will appear stating that the assay requires an update to the scaling factor. Figure 4-3 shows the Save to Instrument window for entering a scaling factor.

Figure 4-3 Save to Instrument Window: Entering Scaling Factor

8. For assays that require updating the standard values with each run, enter the value for each standard in the appropriate field. See Figure 4-4. The default values in the assay file will not be affected.

9. For any assay that requires an update to a control range with each run (Run Time Variable, RTV), select the control to be edited and then select Edit. Enter the new range. Select OK when done. Repeat for any remaining controls. Figure 4-4 shows an assay requiring standard value and control range updates.

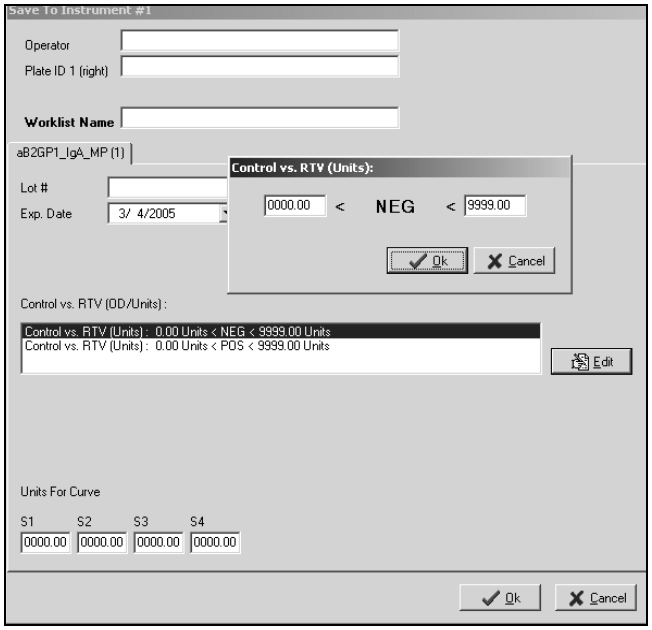

Figure 4-4 Save to Instrument Window: Assay with RTV Controls

10. Select OK when finished.

4.4 Workstation Setup and Completing the Sample Run

1. Perform all Daily Startup Maintenance if this is the first run of the day. This includes Probe Tip Cleaning, Alignment and Priming. Refer to Section 5. For all subsequent runs with the same plate type as previous run, only priming of new wash solution is needed. Operator must realign the Workstation if a different plate type is used.

2. Fill the Wash 1 bottle with the appropriate solution. Perform a prime of the Wash 1 line. Refer to Section 5.9. If using two wash solutions, both wash lines must be primed. The PhD main window will indicate which wash bottle will be used for each test. This information is found under the reagent rack area of the window.

3. Place all reagents, calibrator(s), controls and patient samples on the workstation as indicated on the main window. Verify that there are no bubbles or film across the top of any component or sample.

4. Place the microplate(s) onto the plate platform. The microplate is positioned with A1 in the front right corner. Refer to the main window for proper orientation.

5. Close the workstation cover.

NOTE: *During a sample run, press the pause (-) key on the Numeric Keypad before opening the PhD Workstation door.*

6. Select 5 (Select and Run Worklist) on the workstation keypad. Use the "+" and "-" keys on the keypad to locate the appropriate file if it does not appear as the first worklist on the screen. Select Enter to begin the run.

NOTE: *If an insufficient sample volume is encountered, the workstation will beep to allow the user to either skip the sample or add more volume and retry. If an insufficient reagent volume is encountered, the workstation will beep to allow the user to add more volume and retry. Reagents cannot be skipped.*

7. If all samples and components are present in sufficient volume, the workstation will complete the programmed sample run without interruption until the beep sounds at the end of the run. Select any key on the keypad to silence the workstation. This will send a message to the PhD software to move the .wel file from the ND directory for that workstation into the Done folder of that same directory.

8. Select Read Plate from the PhD main menu. Select the Instrument number. Select the worklist name from the Done folder.

9. Enter a plate ID if required. Remove the first plate from the workstation. Turn the plate so that the A1 position is at the back left. Place the plate on the reader, with A1 to the back left, when prompted. For a second plate, enter the second plate ID (if required) and place the second plate on the reader, with A1 to the back left, when prompted.

NOTE: *The microplate should be read within the time specified by the assay manufacturer.*

10. The results will appear on the screen when reading is complete. Each assay will have its own report. Select the first assay tab to review the results of the first assay. Select Print to print the results. For assays that have generated a standard curve, select Curve to view and print the curve. Repeat for any remaining assays.

11. Perform PhD Shutdown Maintenance. Refer to Section 5.

4.5 LIS Setup and Query

4.5.1 Setup

The LIS Setup window will appear after selecting Setup from the LIS menu.

The LIS Setup window contains four sections (Figure 4-5).

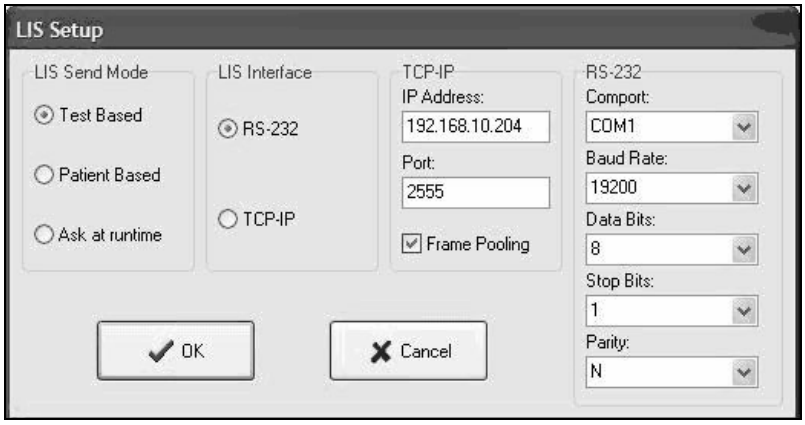

Figure 4-5 LIS Setup

1. LIS Send Mode: The three options (Test Based, Patient Based and Ask at runtime) are only applicable to bi-directional LIS interface for PhD EIA software. For additional details on LIS Send Mode, refer to Appendix F.

   - **LIS Send Mode/Test Based option.** Test-based transmissions have the following format:

     --All Controls for Test 1
     --Patient(1) results for Test 1
     --Patient(2) results for Test 1
     . . . . .
     --All Controls for Test 2
     --Patient(1) results for Test 2
     --Patient(2) results for Test 2

   - **LIS Send Mode/Patient Based option.** Patient-based transmissions have the following format:

     --All Controls for Test 1
     --Patient(1) results for Test 1 **and any other Test results for Patient(1)**
     --Patient(2) results for Test 1 **and any other Test results for Patient(2)**
     . . . . .
     --All Controls for Test 2
     --Patient(M) results for Test 2 **if this patient had no results on Test 1**

. . . . .
--All Controls for Test 3
--Patient(N) results for Test 3 **if this patient had no results on Test 1 or Test 2**

- **LIS Send Mode/Ask at runtime option:** If this option is selected, the user will be prompted to select either Patient-based or Test-Based before results are transmitted to the LIS.

2. **LIS Interface:** Allows the user to designate the appropriate LIS connection (RS-232 (serial port) or TCP/IP).
3. **TCP-IP:** IP Address and Port.

   Frame Pooling may be selected or cancelled for both TCP-IP and RS-232 options. Frame Pooling means that all intermediate frames (starting with the Header frame to the last frame, including the Terminator frame) are pooled and sent as one TCP Packet. This results in a much faster performance. Frame pooling is selected as the default. If Frame Pooling is not selected, every frame is sent as a separate TCP Packet.
4. **RS-232:** For this option, the user must set the following communication parameters.

   Comport: COM1 or COM3

   Baud Rate: 110, 300, 600, 1200, 2400, 4800, 9600, 14400, 19200, 38400, 56000, 57600 or 115200

   Data Bits: 5, 6, 7 or 8

   Stop Bits: 1, 1.5 or 2

   Parity: N, O, E, M or S

Click OK after completing the LIS option selections.

NOTE: *Refer to Appendix F for additional information on PhD LIS Specifications.*
Consult your LIS provider for information on configuring the appropriate RS-232 or TCP/IP connection for your LIS.

4.5.2 Query LIS

The LIS menu allows the user to query the LIS by selecting Query LIS or by pressing the shortcut key F12 (Figure 4-6).

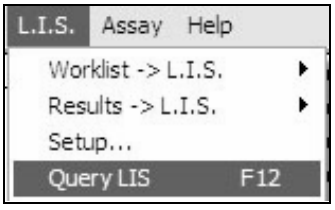

Figure 4-6 Query LIS Command

To send a query to the LIS for retrieving the tests ordered for each sample ID, press F12 (Query LIS) after all sample ID numbers have been entered manually or by barcode scanner. The PhD software will confirm query response by adding ordered assay(s) to the worklist and responding with a beep.

Based on the response from the LIS, the ordered tests are automatically selected in the worklist for the sample IDs. Information on each sample ID can be displayed by clicking the mouse pointer on the position number in the Sample List. The information will indicate if assays were successfully added to the worklist. The color of the position number may change from gray to green or blue.

A color change from gray to blue may indicate one of the following outcomes:

- The selected test is not ordered for the sample ID. For this situation, the user may delete the sample from the worklist/ sample rack or manually select to run the test.
- The selected test ordered for sample ID cannot be added to the run due to limited space. For this situation, the user cannot add the assay to the run unless the test is selected for the second microplate. The user may delete the sample from the worklist/sample rack.
- The sample ID is not recognized by the LIS. For this situation, the user can delete the sample from worklist/sample rack or manually select to run the assay.

A color change from gray to **green** may indicate a combination of outcomes. For example, one assay in the worklist is ordered, but another assay is not ordered. Additional information can be obtained by clicking the mouse pointer on the green position number.

5 Maintenance

All maintenance procedures described in this manual can be safely performed by properly trained personnel.

CAUTION: Maintenance not covered in this manual should be performed only by a qualified Bio-Rad representative.

5.1 Daily Startup Maintenance

It is recommended that Daily Startup Maintenance be performed in the order listed below.

- ❑ Turn on the PhD Workstation and observe that the self tests pass.
- ❑ Clean probe tip tubes internally with the white stylet and externally with isopropyl alcohol (see Section 5.7).
- ❑ Perform the alignment procedure (see Section 5.8) if the workstation was shut down the previous day.
- ❑ Prime the system with wash buffer for 9 cycles (see Section 5.9). If two wash buffers will be used, both must be thoroughly primed.
- ❑ During the priming cycle, check all visible tubing and couplings for leaks, salt crystals or visible damage.

5.2 Daily Shutdown Maintenance

- ❑ Thoroughly prime Wash 1 with deionized water for 9 cycles. If Wash 2 is in use and has wash solution in the line, perform a 9-cycle prime on Wash 2.

- ❑ Empty the waste container. Treat and dispose of waste as directed by laboratory safety guidelines and/or local ordinance.
- ❑ Wipe up any spills using a soft disposable towel or tissue and appropriate cleaning solution.
- ❑ Turn off the PhD computer. It is not necessary to turn the workstation off daily, but if it is not to be used for more than 24 hours, it is recommended to turn it off.

5.3 Weekly Maintenance

- ❑ Perform an acid flush (see Section 5.10).
- ❑ Clean the entire probe (see Section 5.11).
- ❑ Wipe down the inside of the PhD Workstation with isopropyl alcohol.
- ❑ Remove and clean the sample racks with mild detergent and water.

NOTE: *Do not use Acetone on the cover.*

- ❑ For the Model PR3100-RC Microplate Reader only: Clean the cover and plate transport area of the microplate reader with a mild detergent.

5.4 Monthly Maintenance

- ❑ Inspect the probe tip for damage.
- ❑ Disinfect the exterior surfaces of the workstation with 10% bleach solution (0.5% sodium hypochlorite). Wipe off all residual bleach solution with a towel or cloth dampened with deionized water.
- ❑ Clean the Z-rail of the probe holder (see Section 5.12).

5.5 Quarterly Maintenance

- ❑ Replace wash cup, REF 426-0008 (see Section 5.14).
- ❑ Check all tubing for wear and secure attachments.
- ❑ Replace the peristaltic pump tubing, REF 426-0067, package of 10 (see Section 5.15).
- ❑ Perform a Precision Check (see Section 5.13).

5.6 Biannual Maintenance: PR3100-RC Microplate Reader

- ❑ Remove the filter cartridge from the reader and clean the filters using an optical cleaning solution. Lens tissue is recommended. (See Section 5.16.)

5.7 Daily Probe Tip Cleaning

CAUTION: Be careful when using the white stylet to clean the probe tip. The stylet is longer than the metal probe tubes and could puncture the silicone tubing if it is rapidly inserted from the bottom of the probe.

WARNING: Use caution when handling the probe tip to avoid injury. Disposable gloves should be worn when cleaning the probe tip.

1. Remove the probe tip from the magnet at the end of the probe holder. It is not necessary to disconnect the tubing or the cables. The workstation power can be on or off to perform this procedure.

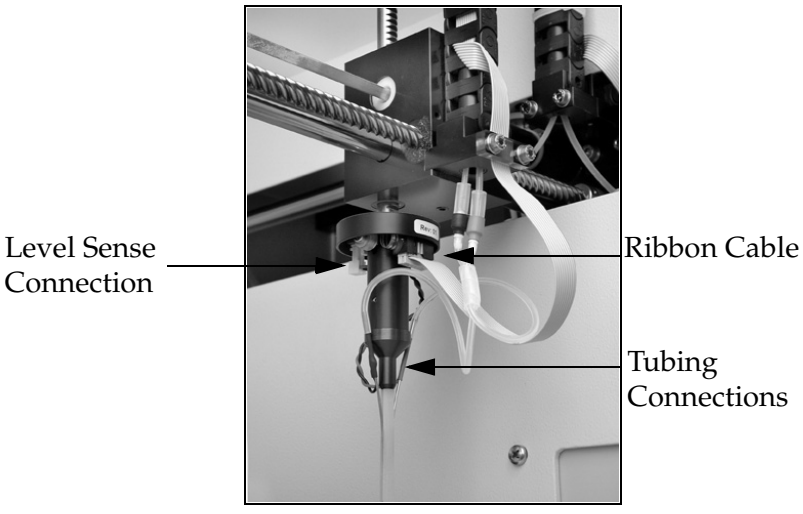

Figure 5-1 Probe Holder and Probe Tip

2. Gently insert the white stylet into the bottom of the aspiration/ dispense probe tube. Gently slide the stylet back and forth until no binding or sticking is felt. If the stylet cannot be easily inserted into the probe tube, proceed to the Complete Probe Tip Cleaning procedure (Section 5.11). Repeat for the waste tube.
3. Wipe the outside of the probe tubes with isopropyl alcohol.

5.8 Alignment Procedure

The alignment procedure must be performed whenever the workstation is turned on.

5.8.1 Materials Required

- One or two empty microplates. The plates should be the same type used when performing a sample run.
- One empty reagent bottle
- Four sample tubes. The tubes should be the same type used when performing a sample run.

5.8.2 Alignment Procedure

WARNING: Use extreme caution if performing the alignment with the workstation cover open, as the probe tip moves automatically when each alignment position is accepted.

1. Place the two empty microplates into position on the microplate platform with A1 in the front, right corner. If only one microplate will be used, place it on the right side of the platform.
2. Place the reagent bottle into the reagent rack in the front right position.
3. Place the sample tubes in the front right and back left corners of each sample rack. Figure 5-2 shows the alignment positions.

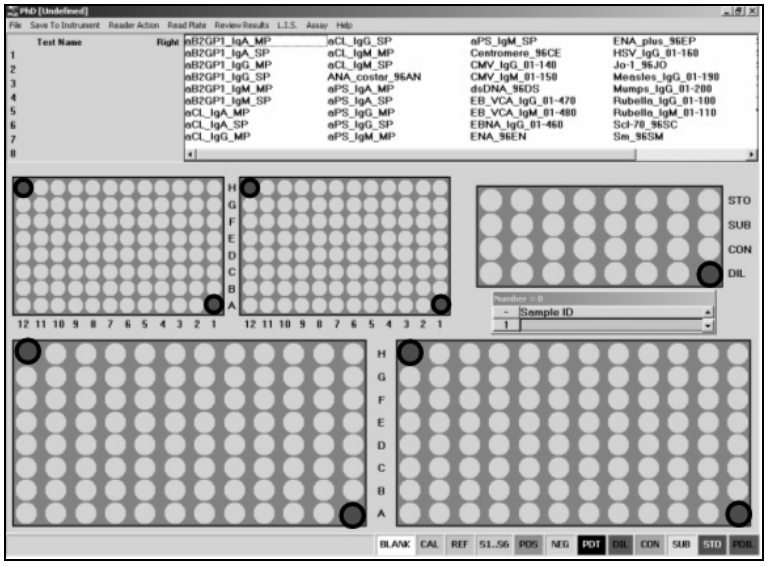

Figure 5-2 Alignment Positions

NOTE: *If only one microplate will be used, it must be moved to the left side of the microplate platform after setting the back well position of the right microplate.*

4. Select 3 (Align) on the workstation keypad to start the alignment. Select Enter after each alignment is set. Once the position is entered, the probe will automatically move to the next position. The probe will move in the following order:

   - Front well position of the right microplate
   - Microplate platform between the microplates to check the Z switch (no alignment required by user)
   - Back well position of the right microplate
   - Reagent position
   - Wash well for alignment by user and then to check Z max
   - Back tube position of the right sample rack
   - Front well position of the left microplate
   - Back well position of the left microplate
   - Back tube position of the left sample rack
   - Front tube position of the left sample rack
   - Front tube position of the right sample rack

5. Select the Num Lock key, on the keypad, to turn off the numbers lock and access the arrows on the keypad. The keys correspond to the following movements, which are also shown on the workstation LCD:

   - 8 to move the probe back (Y alignment)
   - 2 to move the probe forward (Y alignment)
   - 4 to move the probe left (X alignment)
   - 6 to move the probe right (X alignment)

   The "-" and "+" keys move the probe up and down (Z direction). These keys can be used to place the probe tip into the well, bottle or tube in order to better see the alignment position. It is not necessary to align the Z position, only X and Y.

NOTE: *If a forward movement is executed as the last step to align the microplate wells, select the "-" or "+" key once before selecting Enter to set the alignment. Failure to move the probe in a direction other than forward will keep the probe from moving to the next position to complete the alignment.*

6. Center the probe tip over the microplate, wash well and sample rack positions using the keypad. It is critical to center the probe tip accurately in each of these positions. The workstation will calculate the distances of each position between the corner positions for the microplates and sample racks. If the positions are not correctly centered at each end, the calculation may be off, resulting in a probe crash or dispense of fluid between the wells or tubes during a sample run. If the wash well is not centered properly, it may result in carryover due to the probe tip not being washed properly. The reagent bottle is not a centered position, as the bottle is angled in the rack. Position the probe in the bottle so that the probe tip is able to aspirate reagent pooled at the front of the bottle. It is helpful to lower the probe tip into the bottle and move the bottle up and down to verify that the tip will touch the lower inside edge of the bottle (not the side) for low volume reagent. No other part of the probe should touch the bottle.
7. Turn on the Num Lock key on the keypad when all alignments are complete. Remove the microplate(s), reagent bottle and sample tubes.

5.9 Priming the System

Verify that the workstation has been properly aligned before beginning a prime.

1. Fill the wash bottle for either Wash 1 or Wash 2 with the appropriate wash solution or deionized water.

Figure 5-3 Wash and Waste Connections

2. Select either 1 PrimeW1 (to prime Wash 1) or 2 PrimeW2 (to prime Wash 2) on the workstation keypad.

3. Select 9 for the number of cycles.
4. During the priming cycle, verify that no bubbles remain in the external wash tubing and outer harness. Gently tap the external harness tubing while the PhD is priming.
5. Select "*" on the workstation keypad, when the prime is done, to return to the main menu.

5.10 Acid Flush

WARNING: If using 1 N HCl, use caution when handling.

5.10.1 Materials Required

- 15 to 30 mL of 1 N HCl or 1 to 2 bottles of Bio-Rad Stop Solution (Catalog #220SM)
- 2 PhD reagent bottles
- 200 mL deionized water

5.10.2 Procedure

1. If using 1 N HCl, split the acid solution between the two reagent bottles so there is 15 mL in each bottle.
2. Place the Wash 1 tube into one of the bottles containing acid solution (HCl or Stop). Perform a 5-cycle prime. Remove the wash tube from the bottle when the priming is complete and wipe the outside of the glass rod with a lint-free cloth.

NOTE: *It is not necessary to prime Wash 2 if this line is not in use and contains no fluid.*

3. Repeat Step 2 for Wash 2.
4. Replace the acid solution with the container of deionized water and place the wash tube into the container. Perform two 9-cycle primes. If both wash lines were primed with acid, follow with two 9-cycle primes of Wash 2 using the deionized water.
5. Remove the wash tubes from the deionized water. Replace the deionized water with wash solution if a sample run is to be performed.

5.11 Complete Probe Tip Cleaning

WARNING: Use caution when handling the probe tip to avoid injury. Disposable gloves should be worn when cleaning the probe tip.

1. Turn the workstation off and open the front cover.
2. If the probe tip is resting on the reagent rack, gently grasp the Z-rail of the probe holder from the top and lift it up. Using the

other hand, gently grasp the black Delrin block and move the arm assembly to the front of the workstation.

3. Completely remove the probe tip from the probe holder. This includes disconnecting the level sense cable in addition to removing the probe tubing (see Figure 5-1).
4. Gently insert the white stylet into the top of each probe tube. Move the stylet back and forth to clean the tubes.
5. Gently insert the green stylet into the top of each probe tube. This stylet does not fit inside the bottom of the probe tubes. The stylet will stop at the curve of each tube. Do not force it further into the tubes. Move the stylet back and forth to clean the tubes.
6. Repeat Step 4 using the white stylet.
7. Wipe the outside of each probe tube with isopropyl alcohol.
8. Replace the probe tip on the probe holder making sure that the cables are secure and the color-coded tubing is connected properly.
9. If this procedure is performed before the first sample run of the day, prime the system for 9 cycles with wash solution. If this procedure is performed at the end of the day, prime the system for 9 cycles with deionized water as described in Section 5.9.

5.12 Z-Rail Cleaning

5.12.1 Required Materials

- Isopropyl alcohol
- Lint-free tissue or cloth

5.12.2 Procedure

1. Turn the workstation off and open the front cover. Lift the cover up until it rests on the top of the workstation.
2. If the probe tip is resting on the reagent rack, gently grasp the Z-rail of the probe holder from the top and lift it up. Using the other hand, gently grasp the black Delrin block and move the arm assembly to the front of the workstation.
3. Completely remove the probe tip from the probe holder. This includes disconnecting the level sense cable in addition to removing the probe tubing.
4. Moisten the lint-free tissue or cloth with the isopropyl alcohol.
5. Grasp the probe holder at the top and pull it up as far as it will go.

6. Gently wipe the teeth of the Z-rail with the cloth making sure the alcohol gets into the teeth. There should be excess alcohol on the rail, but not enough to drip.
7. Move the probe holder down as far as it will go and repeat Step 6.
8. Move the probe holder up and down several times to ensure that the alcohol gets into the gear teeth in the Delrin block.
9. Move the probe holder up and wipe the excess alcohol off of the rail.
10. Move the probe holder down and wipe off any remaining alcohol.
11. Replace the probe tip, making sure that the color-coded tubing is connected properly.
12. Close the cover.

5.13 Precision Check

5.13.1 Materials Required

- 1 bottle of PhD Yellow Food Coloring (REF 426-0231)
- 1 L of freshly diluted wash buffer containing a surfactant such as Tween. (Bio-Rad recommends using Bio-Rad ANA Wash Concentrate, REF 230AW; dilute 60 mL of wash concentrate into 1 L of deionized water.) Wash buffer must be at room temperature.
- 3 clean 30-mL reagent bottles
- 2 clean sample tubes
- 1 uncoated, flat-bottom microplate (REF 426-0219)
- 20–200 µL micropipette (properly calibrated) with tip
- 10-mL serological pipette
- 1-mL adjustable pipette
- Transfer pipettes

5.13.2 Precision Check Procedure

NOTE: *The concentration of locally purchased yellow food coloring can vary. The results of the precision check may be compromised if the PhD Yellow Food Coloring is not used.*

1. Prepare dilute dye solution.
   - Label one 30-mL reagent bottle “DIL-2”.
   - Accurately dispense 30 mL of wash buffer into the bottle using the serological pipette.
   - Using the micropipette, acccurately dispense 40 µL of the Yellow Food Coloring into the same reagent bottle.
   - Cap the bottle and invert several times to ensure complete mixing. If bubbles form, remove them with a transfer pipette.

2. Prepare concentrated dye solution.
   - Label the second 30-mL reagent botttle “DIL-1”.
   - Accurately dispense 20 mL of wash buffer into the bottle using the serological pipette.
   - Using the 1-mL adjustable pipette, accurately dispense 900 µL of the Yellow Food Coloring into the same reagent bottle.
   - Cap the bottle and invert several times to ensure complete mixing. If bubbles form, remove them with a transfer pipette.
3. Prepare diluent.
   - Label the third 30-mL reagent bottle “Wash”.
   - Fill with wash buffer.
4. Place reagent botttles in reagent rack.
   - Place the diluent bottle, labeled “Wash”, in the first reagent rack position.
   - Place the “DIL-2” bottle in the second reagent rack position (i.e., to the left of “Wash”).
5. Prepare sample tubes.
   - Fill a sample tube with the “DIL-1” from step 2 and place it in the first sample position (front right corner).
   - Fill the second sample tube with wash buffer and place it in the second sample position.

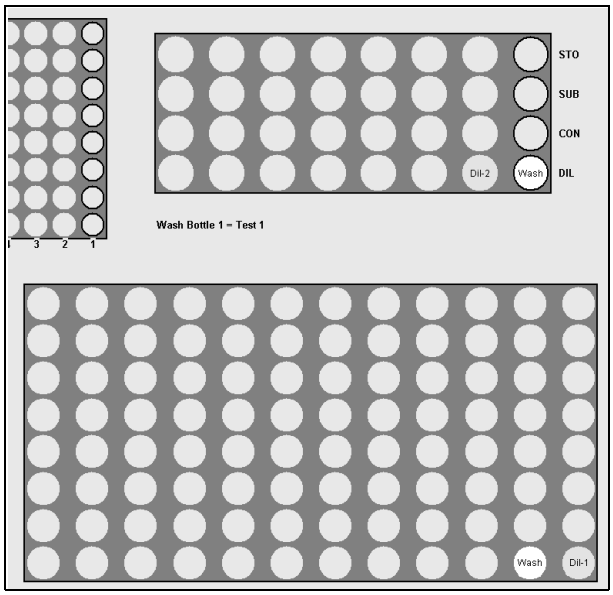

Figure 5-4 Precision Check Layout

6. Thoroughly prime the system (at least 9 times) with wash buffer.
7. Place the uncoated, flat-bottom microplate on the right incubation chamber, with A1 in the front right position. Make sure that the microplate is seated correctly.
8. Using the PhD Numeric Keypad, make sure that the Numbers Lock indicator light is on by pressing the Num Lock key.
9. Press "4" for Precision Check.
10. The Precision Check takes 20–25 minutes to dispense.
11. The workstation will beep when the dispensing is complete. Press any key on the keypad to silence the workstation.
12. From the PhD software main menu, click Reader Action.
13. From the drop-down list, select Precision Check.
14. Remove the microplate and carefully rotate it so that A1 is in the back left corner. Place the microplate on the reader plate support and click OK.
15. Print and file the results.

5.13.3 Specifications

The plate is dispensed as follows:

- Rows A–D use the concentrated dye solution (DIL-1) diluted in wash buffer:

  A: 1 µL

  B: 3 µL

  C: 8 µL

  D: 10 µL

- Rows E–H use Diluted Dye (DIL-2) transferred directly to the microplate well:

  E: 50 µL

  F: 100 µL

  G: 200 µL

  H: 300 µL

The CV for each row must be less than or equal to 6%.

The precision check must be repeated if any row fails this specification. Verify the following before repeating the procedure:

- Check for air in the fluid pathway from the outer wash tube to the probe tip aspiration tube (red coupling).
- Verify that the food coloring concentrations were prepared properly.

If the second precision check fails, contact your regional Bio-Rad representative for further assistance.

NOTE: *If using food coloring purchased locally (not Bio-Rad catalog number 426-0231), the concentration of the undiluted food coloring may not be the same. The volume of food coloring (less concentrated) or wash and deionized water (more concentrated) may require adjustment.*

5.14 Replace the Wash Cup

1. Turn the workstation off and open the front cover. Lift the cover completely up.
2. If the probe tip is resting on the reagent rack, gently grasp the Z-rail of the probe holder from the top and lift it up. Using the other hand, gently grasp the black Delrin block and move the arm assembly away from the wash well.
3. Remove the old wash cup from the reagent rack by pushing the cup up from the bottom until it is dislodged from the silicon O-ring.

4. Dispose of the wash cup according to local or regional requirements for the disposal of biohazardous waste. Also dispose of the O-ring.
5. Place the new cup into position. Hold the cup down and place the new O-ring around the bottom of the cup underneath the top layer of the reagent rack. Make sure that the O-ring is securely in place and that the cup cannot move up or down.

5.15 Replacing the Peristaltic Pump Tubing

1. Turn the workstation off and open the front cover.
2. Open the peristaltic pump door.
3. Remove the old tubing from around the pump rollers and within the clips. Pull the tubing off the ports located to the right of the pump.
4. Place the new pump tubing onto the ports. Make sure they are securely in place.
5. Place the tubing into the clips at the top and bottom of the pump, making sure that the tubing is not stretched. Gently insert the tubing around the rollers of the pump. Turn the pump back and forth to make sure the tubing is properly in place. Figure 5-5 shows proper installation of the pump tubing in the peristaltic pump.

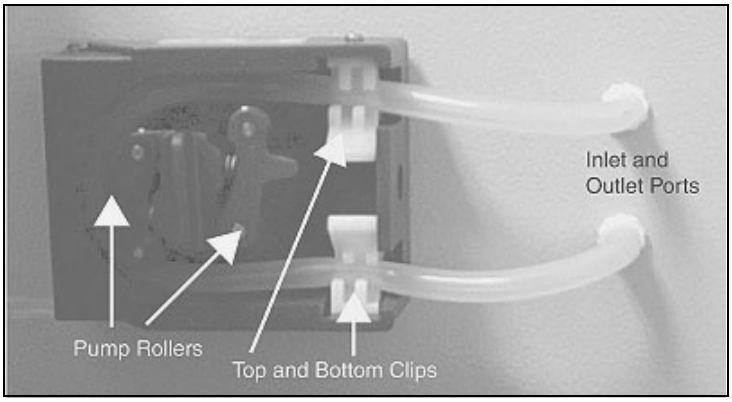

Figure 5-5 Peristaltic Pump

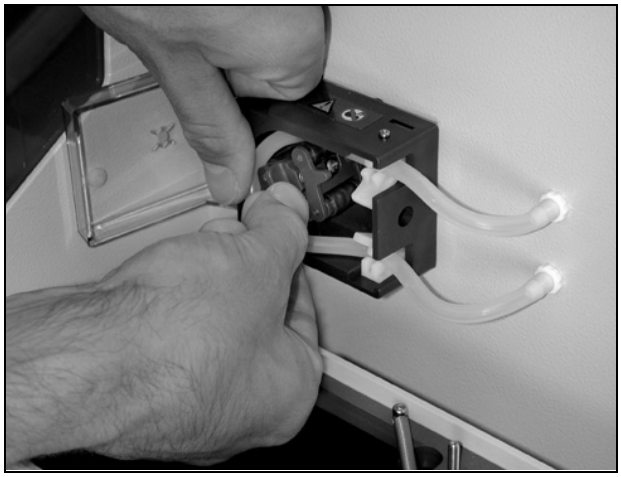

Figure 5-6 Installing the Peristaltic Pump Tubing

6. Close the pump door and the workstation cover.
7. Turn on the power to the workstation and verify that the peristaltic pump moves properly during the self test.

5.16 Cleaning the Model PR3100-RC Filters

1. Verify that the PR3100-RC reader and the PhD computer are turned on.
2. Open the PR3100 Utility icon on the desktop and click the Eject button in the Filter Cartridge section. The filter cartridge will be ejected.
3. Carefully remove the cartridge from the reader.

4. Clean the filters using lint-free lens paper or other non-abrasive lint-free tissue and an optical cleaning solution, without disassembling the filter cartridge.
5. Place the cartridge back in the reader with the CE mark label facing to the left. Gently push it in until resistance is felt. See Figure 5-7.

Figure 5-7 Installing the Reader Filter Cartridge

6. Click the Insert button in the Filter Cartridge section of the PR3100 Utility software application.

5.17 Maintenance Log Sheets

Maintenance log sheets are provided in Appendix D.

5.18 Decontamination and Repacking of the PhD System

Decontamination and repacking of the PhD System should be performed only by an authorized Bio-Rad representative. For safe transport of the PhD, please contact Bio-Rad Technical Service.

6 Troubleshooting

This troubleshooting guide is not all-inclusive. If corrective action is needed that cannot be found in this manual, contact one of the following:

- Bio-Rad Technical Service, for customers in the U.S.A. or Puerto Rico:

  1-800-2BIORAD (24 hours a day, 7 days a week)

- Your regional Bio-Rad office or representative, for customers outside the U.S.A. or Puerto Rico.

6.1 Mechanical Problems

Problem	Possible Cause	Recommended Solution
Wash cup or wells overflow, poor aspiration	Probe tip/tubing plugged	Stylet the probe tips; perform weekly maintenance (Section 5.3).
		Replace Probe Tip Tubing.
	Peristaltic pump tubing not installed correctly.	Exercise, stretch, and reinstall peristaltic pump tubing (Section 5.15).
	Peristaltic pump tubing worn	Replace peristaltic pump tubing (Section 5.15).
	Wash cup worn/damaged	Replace or rotate wash cup (Section 5.14).
	Daily alignments incorrect	Refer to alignment procedure (Section 5.8). Realign PhD.
	Harness tubing clogged	Contact Bio-Rad Technical Service.
The PhD skips reagents or samples	Worklist selected incorrectly	Select #5 for EIA or #6 for IFA; use + or - to scroll through worklists. Select the correct worklist. Repeat run. Delete old worklists.
	Faulty level detection; the PhD appears to be aspirating liquid mid-air	Perform weekly probe cleaning maintenance (Section 5.11). Ensure that the probe tip is completely dry prior to reinstallation.
		Observe fluid lamp; lamp should be lit when not in fluid. Replace probe tip and/or probe holder if faulty.
		Contact Bio-Rad Technical Service.

6.1 Mechanical Problems (continued)

Problem	Possible Cause	Recommended Solution
Probe is dispensing liquid between wells or dispensing outside the well	Dispense and aspirate tubing attached to wrong positions on probe	Inspect tubing; verify that red dispense tubing is to the back and black aspirate tubing is to the front.
	Probe alignment is incorrect	Refer to the alignment procedure (Section 5.8). Realign.
	For IFA, an incorrect worklist is selected, or, for a non-Bio-Rad assay, the slide design is incorrect	Select #6 on the PhD; use + or - to scroll through worklists. Repeat the run. Delete old worklists. Verify that the slide design is correct. Print out slide design and compare to the slide. If assistance is needed with slide design, contact Bio-Rad Technical Service.
	Other mechanical or electrical problem	Contact Bio-Rad Technical Service.
PhD does not dispense liquid	Wash bottle not connected or air in tubing	Check wash fluid level; ensure that the tubing is connected properly to the fluid port on the left side of the PhD. Ensure that the correct wash bottle is designated in the worklist.
	Probe tip tubing installed incorrectly, plugged, or damaged	Inspect tubing; verify that red dispense tubing is to the back and black aspirate tubing is to the front.
	Plugged, pinched, or damaged internal tubing	Contact Bio-Rad Technical Service.
Wash is being aspirated from wrong wash bottle	Incorrect wash bottle selected in worklist setup	Verify that the correct wash bottle is designated in the worklist.
Probe crashing at bottom of tube	Faulty Level Sense	Refer to "Add Reagent" error code (Section 6.2).
	Z-switch failure	Move the probe holder up and down; does the z-switch lamp go on and off? What is the z-switch readout at startup? (Tolerance is 200±50.) Replace probe holder if faulty.

6.1 Mechanical Problems (continued)

Problem	Possible Cause	Recommended Solution
Probe crashing at bottom of microplate well	Normal operation	The PhD usually makes a slight noise when touching the bottom of the well.
	Alignment performed using incorrect well type	Perform alignment using the plate type that corresponds to the assay to be run.
	Z-switch failure	Move the probe holder up and down; does the z-switch lamp go on and off? What is the z-switch readout at startup? (Tolerance is 200±50.) Replace probe holder if faulty.
	Wrong plate bottom defined in assay	If this is a non-Bio-Rad defined assay, check the assay file for plate bottom type.
Probe not moving up or down	Probe holder obstructed	Power off the PhD and manually move the arm up and down, checking for any obstructions. Observe the probe movement during initial self test for any problems. Check the routing of tubing and electrical wires. Remove obstructions.
	Other mechanical or electrical problem	Contact Bio-Rad Technical Service.
Sampling arm not moving during alignment of wells	Alignment steps not performed in proper order	Refer to alignment procedure (Section 5.8). Realign PhD; in the microplate well, make the last forward/backward alignment a backward motion.
Probe tubing "pops" off	Probe or tubing is obstructed	Stylet the probe or replace probe tubing.
	Probe is obstructed with plastic from wash cup	Stylet the probe. Replace wash cup.
PhD will not hold its alignment	External harness connection loose at probe holder	Reseat wire connections.
	PhD is not level or stable	Place the PhD on a solid level surface, free from external vibrations.
	For IFA, the probe tip wires are catching on the slide holder	Reroute wires around the probe; ensure that the wires don't obstruct probe tip motion.
	Other mechanical or electrical problem	Contact Bio-Rad Technical Service.

6.1 Mechanical Problems (continued)

Problem	Possible Cause	Recommended Solution
Probe stalls/hangs up in the middle of a run	PhD is misaligned	Refer to alignment procedure (Section 5.8). Realign PhD.
	Probe arm is obstructed	Power off the PhD and manually move the arm front, back, left, and right checking for any obstructions. Observe the probe movement during initial self test for any problems. Check the routing of tubing and electrical wires. Remove obstructions.
	External Harness is loose	Ensure that the External Harness is connected properly to the probe holder.
	Dirty Probe holder Z-rail	Power off the PhD; the probe holder should gradually fall to the deck on its own. If it doesn't, clean the Z-rail (Section 5.12).
	Other mechanical or electrical problem	Contact Bio-Rad Technical Service.
PhD stops during the run for no apparent reason	The "-" key was accidentally pressed on the Numeric Keypad, activating the Pause command	Press the "-" key again to release the Pause command.
	Worklist selected incorrectly	Select #5 for EIA or #6 for IFA; use + or - to scroll through worklists. Select the correct worklist. Repeat run. Delete old worklists.
PhD dispenses an inaccurate amount of reagent and/or the Precision Check fails	Air bubbles in tubing	Ensure the PhD is fully primed; tap tubing and harness to dislodge air bubbles while priming.
	Probe or tubing is obstructed	Stylet the probe tips. Inspect all tubing for crimps or obstructions. Replace tubing if worn.
	PhD is not on a level or stable surface or PhD is not level or stable	Place the PhD on a solid level surface, free from external vibrations.
	Other mechanical or electrical problem	Contact Bio-Rad Technical Service.

6.2 General Error Messages

Error Message	Possible Cause	Recommended Solution
Add Reagent Please/No Fluid Detected	Insufficient amount of reagent or sample	Add reagent and press "0" to retry.
	DI water is being used in place of reagents or samples during a "dummy run"	Use PBS in place of reagents for a "dummy run".
	Daily probe cleaning with alcohol not performed	Refer to daily maintenance instructions (Section 5.1).
	Z movement is obstructed	Power off the PhD and manually move the probe holder up and down, checking for any tubing or wires in the way. Remove or clear the obstruction.
	Probe tip is dirty, or not connected correctly	Fluid lamp should be on when the probe is not in liquid. Remove probe tip and perform weekly cleaning (Section 5.11). Inspect electrical connections.
Remove drop/ Fluid bridge	Hanging drop on probe	Remove drop. Dry tips with alcohol. Probe tip tubing may have a hole; replace tubing.
	Bent probe tips	Replace probe if tips are bent.
	Body of probe is wet or dirty	Perform weekly probe cleaning maintenance (Section 5.11). Ensure that the probe tip is completely dry prior to reinstallation.
	Wash cup does not fully evacuate, or evacuates slowly	Prime the PhD and visually watch the prime with your eyes level with the wash cup. Refer to Mechanical Problem "Wash cup or wells overflow, poor aspiration" for troubleshooting.
	PhD is misaligned over wash well	Refer to alignment procedure (Section 5.8). Realign PhD.
Z Motor Switch Error/ Z Motor Error	Probe holder Z-rail is contaminated with dust and/ or grease	Clean the Z-rail (Section 5.12).

6.2 General Error Messages (continued)

Error Message	Possible Cause	Recommended Solution
Choice for #6 for IFA worklists is missing from the PhD workstation display	Software not installed correctly	Remove IFA software and reinstall.
Mot Can't Reach Z(1) or Z(2) Position, any key: '8'=back, '2'=front, '5'=center	Probe crashed into the side of the well, sample tube, or reagent bottle due to misalignment	Correct the alignment error by pressing the keys displayed on PhD. Refer to alignment procedure (Section 5.8).
	Bent probe tips	Replace probe tip.
	Z movement is obstructed	Power off the PhD and manually move the probe holder up and down, checking for any tubing or wires in the way. Remove or clear the obstruction.
	Other mechanical or electrical problem	Contact Bio-Rad Technical Service.
Can't go higher OR can't go lower	Z movement is obstructed	Power off the PhD and manually move the probe holder up and down, checking for any tubing or wires in the way. Remove or clear the obstruction.
	Probe holder Z-rail is contaminated with dust and/or grease	Clean the Z-rail (Section 5.12).
	Other mechanical or electrical problem	Contact Bio-Rad Technical Service.
Remove Black Cover	Z movement is obstructed	Power off the PhD and manually move the probe holder up and down, checking for any tubing or wires in the way. Remove or clear the obstruction.
	Plate holder installed incorrectly	Reinstall plate holder. Seat firmly.
	Probe tip damaged	Replace probe.
	Other mechanical or electrical problem	Contact Bio-Rad Technical Service.

6.2 General Error Messages (continued)

Error Message	Possible Cause	Recommended Solution
No link to server	Green Link light on PhD workstation OFF: 1) Cable between PhD, Hub, CPU not connected 2) Hub or CPU not turned on	 1) Reconnect the cables. 2) Cycle power on Hub and CPU.
	Green Link light on PhD workstation ON: 1) PhD folders not shared 2) PhD numbering change was performed incorrectly 3) Other communication problem	 1) Ensure all ND folders are shared. 2) Check the display screen for the PhD workstation number. 3) Contact Bio-Rad Technical Service.

6.3 Software/Peripheral Equipment Problems

Problem	Possible Cause	Recommended Solution
Reader Error: "Check Reader Hardware/cable" (Error occurs immediately when a plate read is initiated.)	The reader or computer power was cycled	Clear error and attempt read again.
	Reader setup is incorrect in the main software	Select "Reader Action", then "Setup", and select the correct reader.
	Reader is not powered on, is disconnected, or is connected incorrectly	Power off reader and computer. Reseat and inspect connections. Power on computer, then reader. Retry read.
	Reader is not functioning	Refer to Section 8 of the PhD Microplate Reader Operation Manual for further troubleshooting.
Reader Error: "Check Reader hardware/cable" (The reader attempts to read the plate but then produces an error.)	Microplate not seated correctly	Attempt a manual read without a microplate to isolate failure.
	Microplate wells not seated correctly	Attempt a manual read without a microplate to isolate failure.
	Reader filter failing	Attempt a manual read with each filter wavelength to isolate failure. Contact Bio-Rad Technical Service.
	Reader malfunction	Refer to Section 8 of the PhD Microplate Reader Operation Manual for further troubleshooting.
PR3100: Reader Problem: Please check the reader connections, Code 0x30000002	Power switched off	Turn on power.
	Power cord unplugged from reader or wall socket	Plug in power cord.
	Serial cable disconnected from reader or computer	Check connections.
	Incorrect serial cable	Ensure the cable is the one supplied with the reader.
PR3100: Reader problem: Please check the reader connections EComPort: Unable to open com port (win error code:5)	PR3100 Utility is running	Go to Desktop and close the PR3100 Utility.

6.3 Software/Peripheral Equipment Problems (continued)

Problem	Possible Cause	Recommended Solution
PR3100: Error reading the microplate Code 0x30000009 or Code 0x30000008	Filters installed incorrectly	Clean filters with lens paper. Reinstall filters using the PR3100 Utility.
	Lamp is failing or has slipped out of housing (PR3100 and BioTek ELx800 only)	Reseat lamp. Refer to lamp replacement procedure in PR3100/BioTek ELx800 Reader Manual.
	Reader malfunction	Contact Bio-Rad Technical Service.
Error: "XXX" filter not in reader	Assay file programmed with a filter not available in that reader	If the reader has been recently installed, it may have a different filter setup from previous model. Verify that the correct reader is selected in Reader Action/Setup. Select the correct filter in the assay file program.
The printer does not print	Printer turned off, out of paper/ink, or paper jam	Check printer status lights for errors.
	Printer not connected to computer	Check connections between printer and computer.
	Printer driver not correct or not available	At computer, select Start/ Settings/Printers. Ensure the correct printer is displayed. If not, select "Add Printer".
Numeric keypad does not work	Num Lock is off	Turn on Num Lock.
	Keypad not connected or connected incorrectly	Check connection between keypad and PhD.

6.4 EIA Assay Result Problems

Problem	Possible Cause	Recommended Solution
Low ODs for Calibrators/Controls	Reagents not at room temperature	Ensure all kit components are at room temperature prior to start.
	Calibrators and controls not mixed	Mix calibrators/controls by gently inverting prior to run.
	Incorrect wash solution used	Verify that the correct wash is selected and is adequately primed through the lines.
	Reagents from different kits or lots mixed	Do not interchange lot-matched reagents.
	Reagent bottles contaminated	Discard reagent bottles. Do not reuse PhD bottles with different reagents. Reagents may be compromised by residual reagent in the bottle, especially interaction of conjugate and substrate.
	Residual reagent in the PhD reagent bottles saved for reuse	Do not reuse reagents that have been run on the PhD. Aliquot only the amount needed for the run, plus 1.5 mL dead volume. Discard any remaining reagent after run.
	Bleach contamination in PhD or reagent bottles	Discard bleach-contaminated reagent bottles or run warm tap water through PhD 18 times, then prime with DI water 18 times. **Bleach is not approved for use with the PhD or reagent bottles.**
	Other PhD or chemistry malfunction	Perform precision check and review results (Section 5.13). Verify reagent storage follows the package insert. Inspect the microplate pouch. Run kit manually for comparison. If kit fails manually, contact Bio-Rad Technical Service.
	Contamination of fluidic lines	Stop Solution should be flushed through the lines weekly (Section 5.10). Visually inspect tubing, especially near fittings, for black particulate. If microbial growth is suspected, contact Bio-Rad Technical Service.

6.4 EIA Assay Result Problems (continued)

Problem	Possible Cause	Recommended Solution
High ODs for Calibrators/Controls	Reagent bottles contaminated	Discard reagent bottles. Do not reuse PhD bottles with different reagents. Reagents may be compromised by residual reagent in the bottle, especially interaction of conjugate and substrate.
	Residual reagent in the PhD reagent bottles saved for reuse	Do not reuse reagents that have been run on the PhD. Aliquot only the amount needed for the run, plus 1.5 mL dead volume. Discard any remaining reagent after run.
High blank ODs	Poor aspiration	Verify correct alignment to wash cup. Remove and replace peristaltic pump tubing. Stretch and exercise tubing before reseating (Section 5.15).
	Poor aspiration, probe tip plugged	Perform weekly probe cleaning maintenance (Section 5.11).
	Poor wash, daily alignment not performed	The PhD alignment must be performed each time the PhD is powered off.
	Poor wash, alignment performed using different plate type	Align using the microplate type that corresponds to the assay to be run.
	Contamination of fluidic lines	Stop Solution should be flushed through the lines weekly. Visually inspect tubing, especially near fittings, for black particulate. If microbial growth is suspected, contact Bio-Rad Technical Service.
	Plate read backwards	Ensure the plate is inserted with A1 corner at upper left.

6.4 EIA Assay Result Problems (continued)

Problem	Possible Cause	Recommended Solution
High CVs for Calibrators/Controls	PhD not fully primed, or air bubbles in the tubing	Ensure PhD is fully primed; tap tubing and harness to dislodge air bubbles while priming.
	Bubble on surface of calibrator or control vial	Inspect vials just prior to running the PhD.
	Probe tip dirty	Clean probe tip using stylets.
	Calibrators and controls not mixed	Mix calibrators/controls by gently inverting prior to run.
	Reagents not at room temperature	Ensure all kit components are at room temperature prior to start.
	Other PhD or chemistry malfunction	Perform precision check and review results (Section 5.13). If precision check fails, contact Bio-Rad Technical Service. Run kit manually for comparison. If kit fails manually, contact Bio-Rad Technical Service.

Appendix A: Parts List

A.1 Replacement Parts Available from Bio-Rad

Contact your Bio-Rad representative for additional information.

Catalog Number	Description
426-0008	PhD/Star Wash Cup and O Ring
426-0016	PhD Probe Tip
426-0017	PhD Probe Holder without Probe Tip
426-0023	PhD Wash Tube
426-0067	PhD/Star Peristaltic Pump Tube, 10 pack
426-0102	PhD Wash Bottle w/Cap
426-0103	PhD Waste Bottle w/Cap
426-0106	PhD White Stylet
426-0107	PhD Green Stylet
426-0120	PhD/Star Outer Waste Tube
426-0121	PhD Silicone Probe Tip Tube
426-0122	PhD/Star Couplings Probe Tip
426-0128	PhD/Star Sample Rack, 14.5 mm
426-0129	PhD/Star Sample Rack, 15.5 mm
426-0130	PhD/Star Sample Rack, 16.5 mm
426-0137	PhD Control/Calibrator Rack Adapter
426-0220	PhD Clear Reagent Bottle, 30 mL, 24/pack

Catalog Number	Description
426-0221	PhD Amber Reagent Bottle, 30 mL, 8/pack
426-0231	PhD Yellow Food Coloring
220SM	Stop Solution, 15 mL
230AW	Wash Concentrate, 60 mL

Appendix B: Packing List

B.1 Workstation

_____	1	PhD Workstation, Serial Number: _____
_____	2	Glass Wash Tubes
_____	1	Silicon Waste Tube
_____	2	Probe Tubes (connected at outer harness of workstation)
_____	1	Peristaltic Pump Tubing (connected to ports on workstation)
_____	1	Probe
_____	1	Probe Holder
_____	1	Numeric Keypad
_____	1	Wash Cup (in reagent rack)
_____	2	13.5 mm Sample Racks
_____	1	Power Cord
_____	1	Side shipping screw and elastic band (installed on workstation)

B.2 Peripherals

_____	1	Computer (Brand & Model:_____Serial # _____)
_____	1	Computer Keyboard
_____	1	Monitor (Brand & Model:_____Serial # _____)
_____	1	Printer (Brand & Model:_____Serial # _____)
_____	1	Microplate Reader (Brand & Model:_____Serial # _____)
_____	1	Barcode Scanner (Brand & Model: _____Serial # _____)
_____	1	Barcode Scanner Cable
_____	1	UPS (for 426-0000 only) (Brand & Model:_____ Serial # _____)
_____	1	Ethernet Hub (for 426-0000 only)(Brand & Model: _____ Serial # _____)
_____	1	Surge Protector (for 426-0000 only)

B.3 Other

_____	1	Category 5 Patch Cable, 3 ft
_____	1	Category 5 Patch Cable, 14 ft
_____	1	Printer Cable
_____	1	Printer Power Adapter
_____	8	30 mL amber reagent bottles w/caps
_____	24	30 mL clear reagent bottles w/caps
_____	2	1 L wash bottles w/caps
_____	1	2 L waste bottle w/cap
_____	12	13 x 75 mm test tubes
_____	1	Yellow Food Coloring Dye
_____	1	Uncoated microwell plate
_____	1	White stylet
_____	1	Green stylet
_____	1	Operation Manual
_____	1	PhD Software and Methods CD (current version)
_____	1	Packing list
_____	___	_____
_____	___	_____
_____	___	_____

Packed by: _____ Sent to:_____

Approved by:_____ Date: _____

Appendix C: Maintenance Log Sheets

Daily/Weekly Maintenance Log Sheet

Month:_____ Year: _____ PhD Serial #: _____

MAINTENANCE CHECKLISTS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
DAILY STARTUP																															
Observe self tests pass																															
Clean probe internally with white stylet; clean externally with isopropyl alcohol																															
Perform alignment procedure																															
Prime system with wash buffer																															
Check visible tubing couplings for leaks, salt crystals or damage																															
DAILY SHUTDOWN																															
Prime system with DI water																															
Empty waste container																															
Wipe up all spills																															
Turn workstation off																															
WEEKLY CHECKLIST																															
Perform acid flush																															
Clean entire probe																															
Wipe workstation with isopropyl alcohol																															
Remove and clean sample racks																															

Monthly, Quarterly and Biannual Maintenance Log Sheet

Year: _____ PhD Serial #: _____

MAINTENANCE CHECKLISTS	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
MONTHLY CHECKLIST												
Inspect probe tip for damage												
Disinfect exterior surfaces												
Clean the Z-rail of the probe holder												
QUARTERLY CHECKLIST												
Replace wash cup												
Check all tubing for wear and secure attachments												
Replace the peristaltic pump tubing												
Perform precision check												
BIANNUAL CHECKLIST												
Remove filter cartridge from reader and clean filters												

Appendix D: Method Editing

D.1 Introduction

NOTE: *Prior to reporting results, the user must validate any assay they add to the system. Assays not validated or manufactured by Bio-Rad Laboratories are not eligible for support by Bio-Rad Technical Service.*

Bio-Rad distributed assays have been validated for use on the PhD. These assays are included as part of the PhD menu or are available on the PhD EIA/IFA Software and Methods CD (Catalog No. 426-0246). Validation studies were limited to those assays where the PhD could be programmed to reproduce the manual method instructions. It is the responsibility of each user to verify any method used on the PhD and to determine the performance characteristics of each method used for reporting patient results in their laboratory.

The PhD software contains test parameters for a variety of assays; however, the user can add additional assays to the system by using the Assay option on the main screen. This document is intended to assist the user in the development of these additional assays.

There are two ways to create a new assay. The first is to open a current assay, save it under another name, edit the parameters and save the changes. The second is to create an entirely new assay that is not based on a previous one. This document will describe the latter.

Appendix E describes how to install new Bio-Rad validated assays and updates to current assay files in the PhD menu.

D.2 Overview of the Assay Window

D.2.1 Accessing the Assay Option

1. Open the main window of the PhD software.
2. Select Assay from the menu. The password, seq, is required to open the Assay window. The password is not case sensitive.

Figure D-1 shows the main Assay window. The user defines the test parameters in this window. These parameters include defining an LIS name, plate type, any predilution step, calibrator, standards, controls and sample handling, incubation times and temperatures, wash steps, and reagent additions.

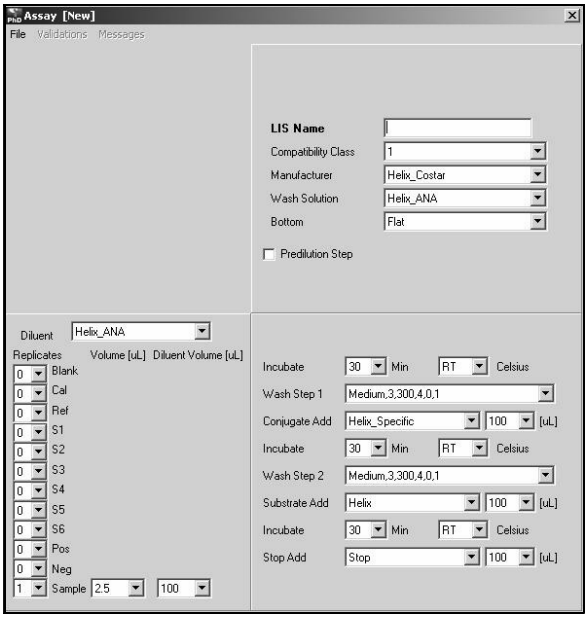

Figure D-1 Main Assay Window

D.2.2 Drop-Down Lists

The main window contains drop-down lists with several predefined options. One of these options is selected as the default setting. These lists can be displayed by selecting the down arrow for each field (Figure D-2).

The options within these lists can be edited to reflect the user's name or value preferences. Editing of these lists is described in Section D.7.

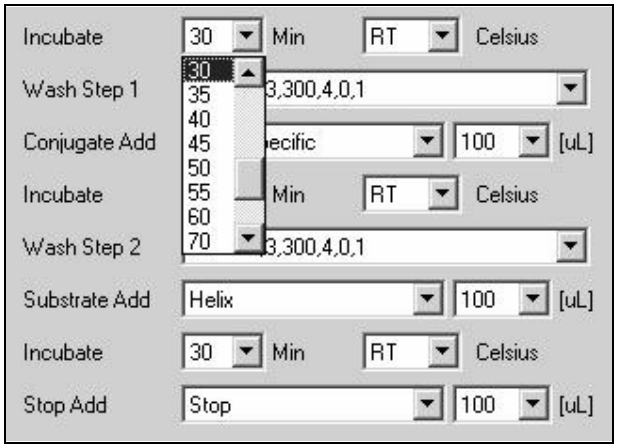

Figure D-2 Example of Drop-Down List

D.2.3 Assay Parameters

LIS Name: Required for transmitting data from the PhD to an LIS.

Compatibility Class: User-defined option for linking assays together. Once an assay is selected for a run, all assays with the same class number will remain as menu options and all other classes will be eliminated.

Manufacturer: Defines the type of microtiter plate to be used.

Wash Solution: Defines the name of the wash solution to be used.

Bottom: Flat or round bottom microtiter wells.

Predilution Step: Defines predilution of calibrator, standards, controls and samples. When this step is selected, the dilution parameters, along with a drop-down list of PRE Diluents will appear on the top left side of the window. An option for incubation time will also appear below the Predilution Step check box (Figure D-3).

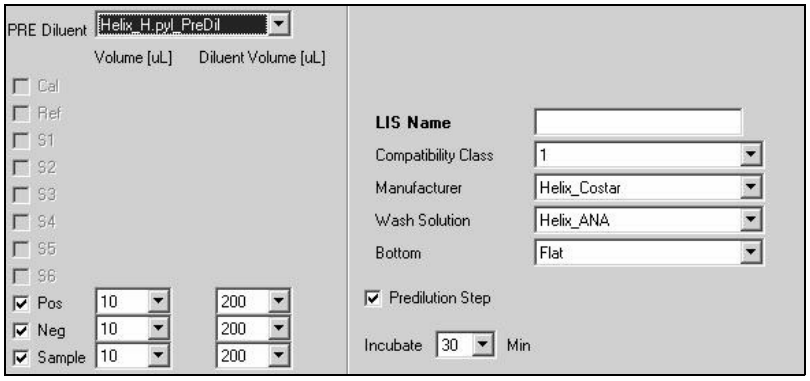

Figure D-3 Predilution Step Window

Diluent: This section of the window is used to define the dispensing parameters for all sample types into the microplate wells. A diluent can also be selected if calibrators, standards, controls or samples are to be diluted directly into the microplate wells. Diluent for any Blank wells is also defined in this field.

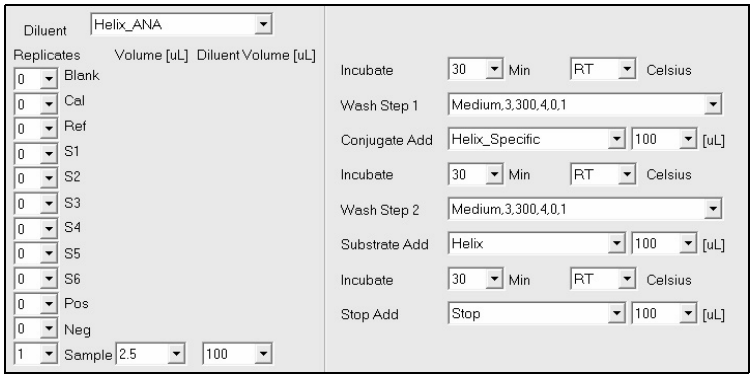

Figure D-4 Diluent Window

Incubate: Defines the incubation time and temperature for the assay. Temperatures can be room temperature (RT) or in the range of 25–40 °C.

Wash Step (1 and 2): The wash step option contains a name and series of numbers. The name reflects the dispense speed. Slow is a dispense speed of 3, Medium is a dispense speed of 4, Fast is a dispense speed of 5 (Figure D-5).

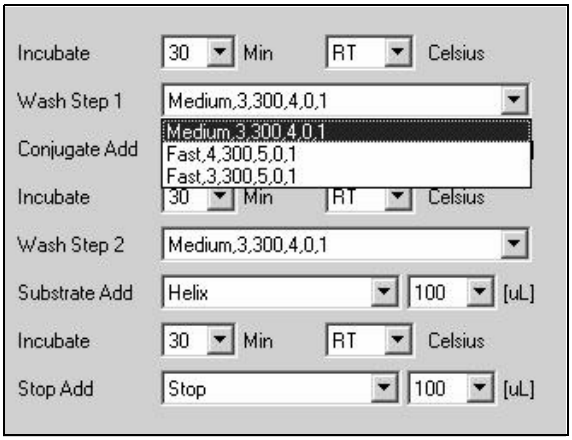

Figure D-5 Wash Step

The numbers that follow the dispense speed name define the following parameters in the order listed:

- Number of wash cycles (1–9)
- Volume of wash (100–400 µL)
- Dispense speed (range is 1–5, slow to fast)
- Defined bottom of the well (0–9 mm). The default is 0, which is the recommended setting. The space between the bottom of the well and the probe is usually 0 mm to ensure complete aspiration of the well.
- Aspiration Needle Position: To position the waste tube at the inside edge of the well for complete aspiration (1). To center the aspiration needle (0).
- Wash Mode: Standard Wash (0); wash volume will remain in wells after the wash cycle. Residual Volume Wash (1); volume remaining in the wells may be defined.
- Defined Residual Volume (0–400 µL, 20-µL increments). Used to minimize the volume remaining in the wells after the wash cycle.
- Additional parameters reserved for future use.

Conjugate, Substrate and Stop Add: Defines the name of each of these reagents and the volumes to be dispensed.

D.3 Creating a New Assay

Use the package insert from the desired kit to program the assay.

Open the PhD main window by double-clicking the icon on the desktop.

Select the Assay option from the banner menu at the top of the window and enter the password, seq, when prompted. The top of the open window will read "Assay [NEW]".

D.3.1 Selecting the Test Parameters

The PhD Software contains predefined options in drop-down lists for programming assay parameters. When programming assays, you must select an option from the drop-down list. Additional options may be created by editing the parameter list. The procedure for creating additions to these lists can be found in Section D.7.

1. Enter an alphanumeric LIS name. The LIS name is a required field. This name must be compatible with the connected LIS system.

2. Determine the Compatibility Class. Define the class number based on the user preference. The class number options are 1–10. For example, all assays with the same incubation time or temperature could be defined as one class, and assays using

different incubation times or temperatures defined as another class. Most Bio-Rad validated assays are currently defined as a Class 1.

Guidelines for determining compatibility include, but are not limited to, the following:

- Assays with the same incubation times and/or temperatures
- Assays with the same Wash Steps defined
- Assays with wash solutions incompatible with other assays

3. Select the Manufacturer of the microplate (see Figure D-1).

4. Select the Wash Solution to be used (see Figure D-1).

5. Select the type of microplate Bottom. Options are flat or round.

6. Select the Predilution Step check box if calibrator, reference, standards, controls or samples require predilution or pretreatment before being dispensed into the microplate. The predilution can be used if a higher dilution ratio cannot be accomplished with the in-well dilution step. Predilutions will be made into sample tubes before being dispensed into the microplate.

   Once the check box is selected, an incubation time option will appear immediately below it. The options are in 1-minute increments between 1 and 10, 5-minute increments between 10 and 60, and 10-minute increments between 60 and 100. The maximum incubation time is 120 minutes and the default is 30 minutes.

   - Select the name of the PRE Diluent.
   - Select the sample types to be prediluted.
   - Select the Volume (µL) of each sample type to be dispensed and the Diluent Volume (µL). The sample volume options are in 0.5-µL increments from 1 to 10 with 10 as the default. The remaining options are in 20-µL increments from 20 to 100. The diluent volume options are in 20 and 50-µL increments from 100 to 1000. The default is 200 µL. **The combined volume of the sample and reagent cannot exceed 1000 µL.**

7. Define the Dispense Parameters in the lower left corner of the window. Select a Diluent if a dilution directly into the microplate is required (see Figure D-4). If no dilution is necessary, leave the field with the default setting. The initial window will show dilution parameters for the sample only. The default is 2.5 µL of Sample and 100 µL of Diluent.

NOTE: *If an existing assay has validation rules defined and the replicate of any sample type (except sample) is changed to 0, a warning message will appear stating that all validation rules and messages are cleared. The user must then redefine the validation rules.*

8. Select the number of Replicates for each sample type. The list of sample types includes blank, calibrator, reference, standards, controls and sample. The number of replicates ranges from 0 to 10, 12 and 14. For sample, the range starts at 1 instead 0. Any sample type not required by the package insert, such as a blank or a reference control, will have a replicate number of 0. Once the number of replicates is selected for Blank, Cal, Ref, S1 to S6, Pos and Neg, the sample dilution volume boxes will appear beside the selected sample type (Figure D-6).

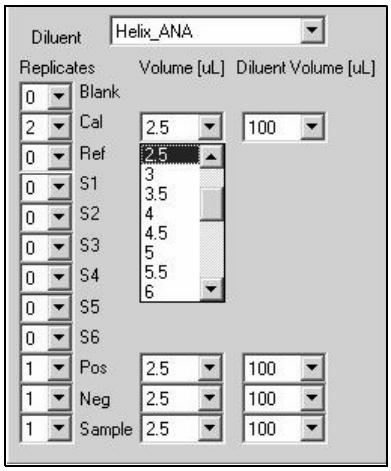

Figure D-6 Dilution Parameters

9. Select the Volume (µL) of each sample type to be dispensed and the Diluent Volume (µL) if applicable. If no dilution is required, enter only the Volume (µL) for each sample type and enter 0 for the corresponding Diluent Volume (µL).

   The volumes selected will be aspirated from the sample tube and reagent bottle then placed into the appropriate microplate well. The sample volume options are 0, 1 to 10 (in 0.5-µL increments), and 20 to 100 (in 20-µL increments). The default is 2.5 µL. No drop-down list appears for volume if a Blank sample is selected; only a diluent volume list appears.

   The diluent volume options are as follows:

   0, 50, 60, 80, 100, 120, 140, 150, 160, 180, 200, 220, 240, 250, 260, 280, 300, 320, 340, 350, 360, 380, 400.

   The combined volume of sample and diluent cannot exceed the capacity of the microplate well.

10. Select the first Incubation time and temperature.

    Times range from 1 to 10 minutes in 1-minute increments, 10 to 60 in 5-minute increments, and 60 to 100 in 10-minute

increments. The maximum time is 120 minutes with a default setting of 30 minutes.

Temperature is in Celsius. The default is RT (room temperature). Heated settings range from 25 to 40 in 1-degree increments.

11. Select an option for Wash Step 1 (see Figure D-5). Refer to Section D.2.3 for interpretation of the wash step selections. To further customize Wash Step 1 parameters, right click the Wash Step 1 drop-down arrow and select Edit to access the Edit WashStep window (Figure D-7).

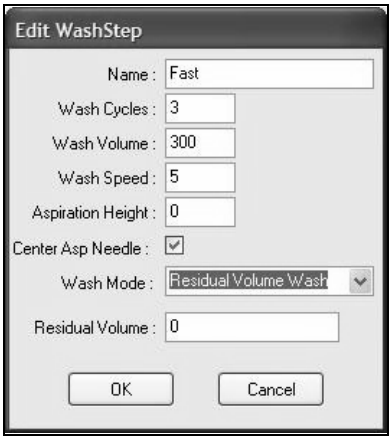

Figure D-7 Edit WashStep Window

In addition to defining the wash parameters for wash cycles, wash volume, wash speed and aspiration height, the user can also define the following parameters in the order listed:

Center Asp Needle:

When selected, aspirates from the center of the well. When not selected, aspirates from the inside edge of the well for more complete aspiration. It is recommended not to select this option, especially when using round-bottom microplates.

Wash Mode:

From the drop-down list, select Standard Wash or Residual Wash. With Standard Wash, the defined wash volume will remain in the wells after the wash cycle. With Residual Volume Wash, the volume remaining in the wells may be reduced by defining Residual Volume (0–400 µL, 20-µL increments).

12. Select a Conjugate from the drop-down list and its dispense volume (µL).

The options for conjugate volume are 100, 200, 300 and 400 µL.

13. Select the second Incubation time and temperature. Refer to Section D.3.1 step 8 for the list of options.

14. Select an option for Wash Step 2. Refer to Section D.2.3 for interpretation of the wash step selections. To further customize Wash Step 2 parameters, refer to Section D.3.1 step 9.

15. Select a Substrate from the drop-down list and its dispense volume (µL).

    The options for substrate volume are 50, 100, 200, 300 and 400 µL.

16. Select the third Incubation time and temperature. Refer to Section D.3.1 step 10 for the list of options.

17. Select a Stop Solution from the drop-down list and its dispense volume (µL).

    The options for stop volume are 20 and 50-µL increments from 50 to 400 µL.

NOTE: *It is recommended that the file be saved after this window has been programmed.*

18. Select FILE/Save As from the menu bar. The folder in which to save the file is entitled ASA and the file type is Assay. Name the assay and select Save. The title "Assay [NEW]" at the top of the window will change to the saved file name.

    The Bio-Rad naming conventions for a Bio-Rad assay file do not allow the use of spaces or special characters and are as follows:

    - Assay name_isotype (e.g., ASCA_IgG)
    - Assay name_isotype_single-point or multi-point (e.g., aCL_IgG_SP or aCL_IgG_MP)
    - Assay name_kit catalog number (e.g., dsDNA_96DS)

19. Proceed to Section D.4 (Validations) when finished selecting all test parameters.

D.4 Validations

D.4.1 Setting the Wavelength and Blank Subtraction

Select the Validations menu from the menu bar. This window allows the programmer to define the wavelength(s) of the assay, select the units to be used, and select the formula for calculating results and validation criteria for controls (Figure D-8). An option for Blank Subtraction is also located on this window.

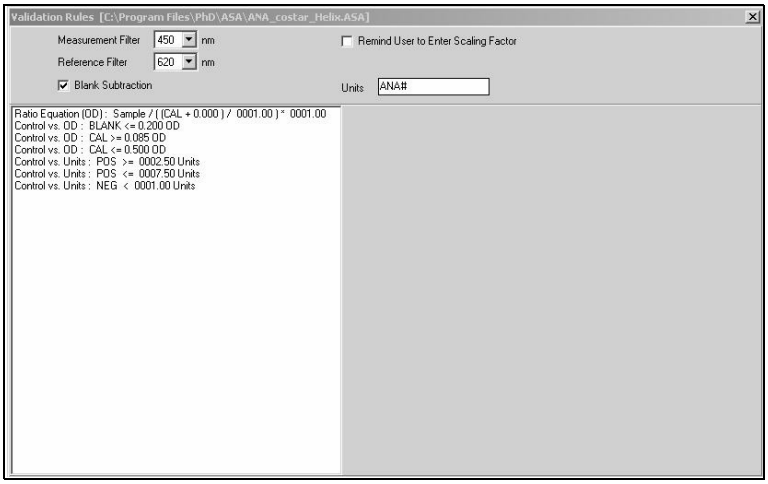

Figure D-8 Example of Validation Rules Window

1. Select the Measurement Filter wavelength from the drop-down list. Options are 405, 450, 490, 550, 620 and 630 nm. The default setting is 405 nm.
2. If applicable, select the Reference Filter wavelength from the drop-down list. Options are 405, 450, 490, 550, 620, 630 nm and NA if no reference wavelength is indicated.

   The Anthos 2010 and PR3100 readers support the following filters: 405, 450, 550 and 620 nm. The BioTek ELx800 supports the following filters: 405, 450, 550 and 630 nm.
3. Select the box for Blank Subtraction if the absorbance of the Blank well is to be subtracted from all wells before result calculation.

D.4.2 Selecting the Result Calculation Option

NOTE: *Once an option for evaluating data (qualitative or quantitative) has been defined and the Validation Rules window is no longer open, the user cannot edit the parameters (e.g., control type, mathematical symbol, etc.) that were set. The result calculation option must be deleted and then completely redefined.*

NOTE: *All control types (e.g., calibrators and controls) previously defined in the main assay window will be listed for use in calculating results.*

NOTE: *The PhD software will allow the user to choose only one option. If a second option is selected, it will automatically replace the one previously defined.*

Right click in the section immediately below the Blank Subtraction check box to display the options for calculating sample results (Figure D-9). There are three options for qualitative assays and six curve fit options for quantitative or semi-quantitative assays. Once an option is selected, the information section appears on the right side of the window.

Once an equation for calculating sample results has been selected, the option for Units will appear in the top right portion of the window. Enter the units to be used (e.g., mg/dL). The only equation that does not show this field is Cutoff (returns no units).

Select OK once an equation or curve is defined. The selected equation or curve will appear in the section immediately below the Blank Subtraction box. The curve fit will include the standards values along with the type of curve to be used.

The following sections provide specific details on selecting result calculation options.

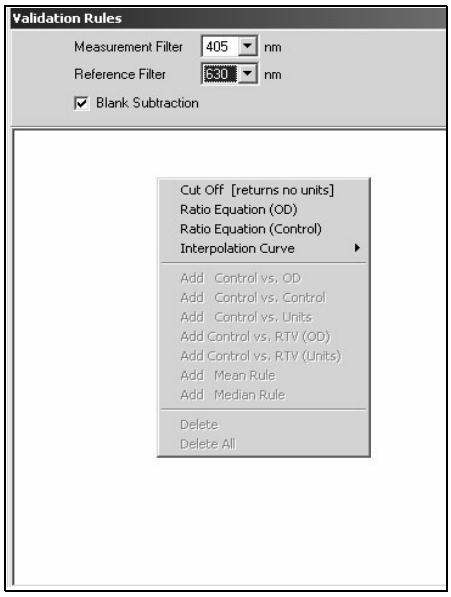

Figure D-9 Equation Options

NOTE: *The term "control type" is used to define calibrator, reference, standards, positive or negative kit components.*

Equations for Qualitative Assays

1. The **Cut Off (returns no units)** equation calculates results based on the selected control type OD reading (Figure D-10). Units cannot be defined if this option is chosen.

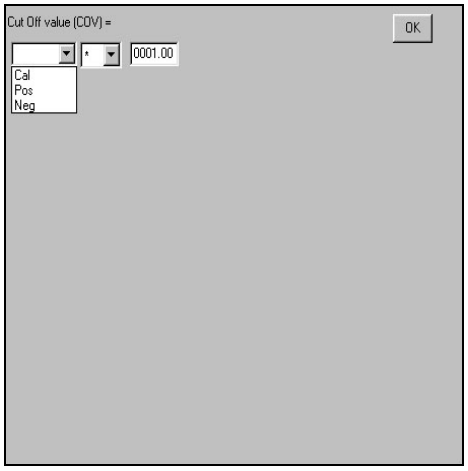

Figure D-10 Cut Off (returns no units) Option

   - Select the control type to be used from the drop-down list.
   - Select a mathematical symbol from the drop-down list of symbols. The options are (*) for multiplication, (+) for addition, (-) for subtraction, and (/) for division.
   - Enter a value in the box following the math option.

2. The **Ratio Equation (OD)** option calculates results based on a ratio of the Sample OD to the OD of the selected control type (Figure D-11).

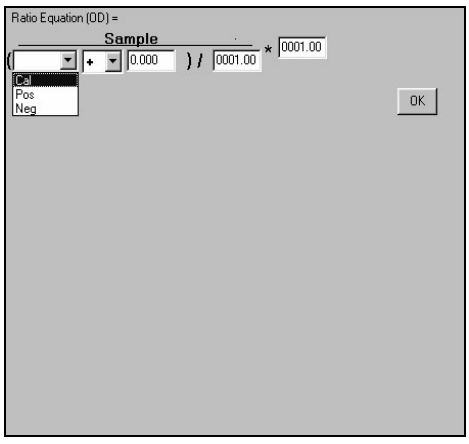

Figure D-11 Ratio Equation (OD)

   - Select the control type to be used from the drop-down list.

- Select addition or subtraction (+ or -) from the drop-down list and enter a value in the box that follows.
- Enter a value in the next box for denominator.
- Enter a scaling factor after the (*) symbol if needed. This scaling factor is set only if the value does not change from lot to lot.

  If the formula is only Sample OD/Control OD, the values will be as follows:

  Sample Type + 0.00/1.00
  Scaling Factor of 1.00

3. The **Ratio Equation (Control)** allows the programmer to use OD readings from two different control types in the denominator, instead of inserting a defined value as in Ratio Equation (OD). (See Figures D-12 and D-13.)

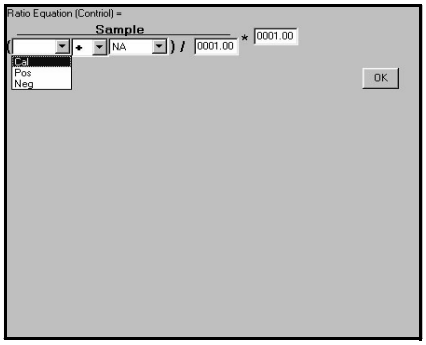

Figure D-12 Ratio Equation (Control), First Denominator Option

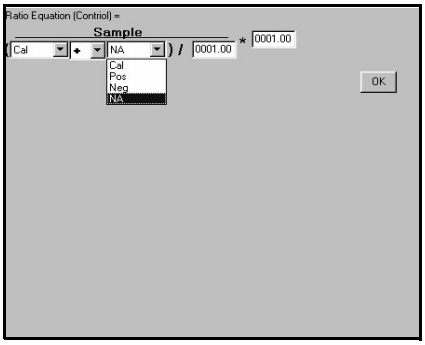

Figure D-13 Ratio Equation (Control), Second Denominator Option

- Select the first control type.
- Select add or subtract (+ or -).
- Select the second control type or NA if a second option is not required.
- Enter a value in the denominator box.
- Enter a scaling factor after the (*) symbol if needed.

Curve Fits for Quantitative and Semi-Quantitative Assays

NOTE: *Curve fitting functions were developed by Brendan Technologies, Inc. and are used in its StatLIA® software.*

The **Interpolation Curve** option is used for quantitative or semi-quantitative assays.

There are six options for defining a standard curve: Point to Point, Cubic Spline, Regression Line, 4-Parameter Logistic, 5-Parameter Logistic, and Logit-Log (Figure D-14).

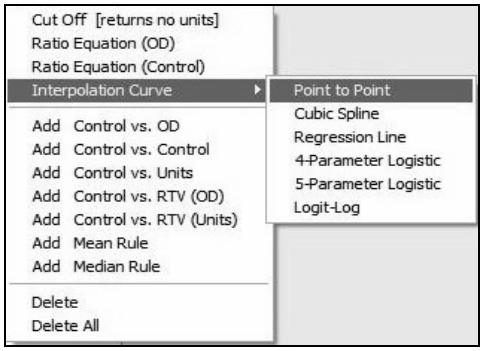

Figure D-14 Interpolation Curve Options

NOTE: *Once an option for evaluating data (qualitative or quantitative) has been defined and the Validation Rules window is no longer open, the user cannot edit the parameters that were set. The result calculation option must be deleted and then completely redefined.*

1. Right click under Blank Subtraction field and select Interpolation Curve. Select a curve fit type to be defined. The section on the right will list all control types previously defined. Select the check boxes next to the standards to be used (Figure D-15).

NOTE: *Control values may be edited during sample run programming.*

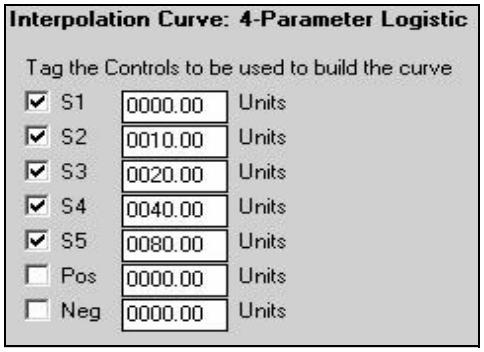

Figure D-15 Interpolation Curve, Selecting the Standards

2. Enter the values for each control (Figure D-15). If the values for the assay will vary from lot to lot, enter 0 for all control values. The user will be prompted to enter the current values during sample run programming when the run is saved to an instrument.

D.4.3 Result Validation Options

Once the Data Analysis option is set, right click in the section of the window below the Blank Subtraction check box to display the Result Validation options (Figure D-16).

There are seven options for setting validation criteria for any control type: Control vs. OD, Control vs. Control, Control vs. Units, Control vs. RTV (OD), Control vs. RTV (Units), Mean Rule and Median Rule.

NOTE: *The term "control type" is used to define calibrator, reference, standards, positive or negative kit components.*

Select one of these options and a window for entering information will appear on the right side of the screen. All control types that were defined in the Main Window will appear in the drop-down menu for Control. Patient sample evaluation criteria are set under the Messages option (Section D.5).

If a Control does not meet the defined criteria, it will be flagged on the final report and the run will fail.

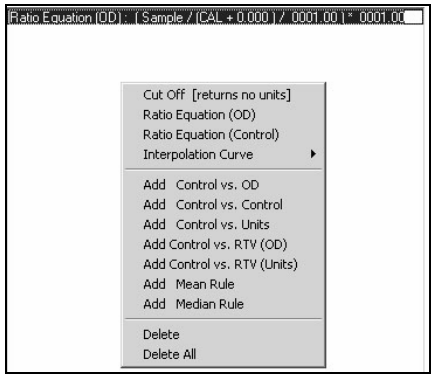

Figure D-16 Result Validation Options

1. **Control vs. OD** defines acceptable OD readings for various Controls. Any or all of the Controls can be defined.
   a. Select the Control from the drop-down list.
   b. Select the option for evaluation. There are four options >, >=, < and <=.
   c. Enter the OD value. In the example in Figure D-17, the Blank must be less than 0.200 to be a valid result.

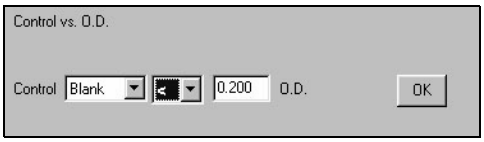

Figure D-17 Control vs. OD

   d. Click OK when complete and then reselect the option to add criteria for other Control types if needed.

2. **Control vs. Control** defines the validation criteria based on two different Control types. The system will evaluate the result from the selected Control by comparing it to the result of the second Control.
   a. Select the first Control from the drop-down list.

b. Select the option for determining the criteria. There are four options >, >=, < and <=.

c. Enter a value for comparing the two controls.

d. Select the second Control type. In the example in Figure D-18, the Positive control must be greater than 5 times the Negative control to be a valid result.

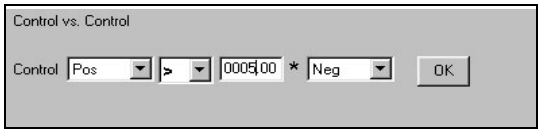

Figure D-18 Control vs. Control

e. Click OK when complete and then reselect the option to add criteria for other Control types if needed.

3. **Control vs. Units** defines the criteria for accepting a control based on the final result (Units).

   a. Select the Control type from the drop-down list.

   b. Select the option for determining the criteria. There are four options >, >=, < and <=.

   c. Enter the Units value in the box. In the example in Figure D-19, the Positive control must be greater than 5 to be valid.

Figure D-19 Control vs. Units

   d. Click OK when complete and then reselect the option to add criteria for other Control types if needed.

4. **Control vs. RTV (OD)** defines Run Time Variables (RTV) for Controls based on the OD. A Run Time Variable is a defined range of values (i.e., lower to upper limit OD range) for a given Control that the user edits at run time. The RTV (OD) is used when a control OD will vary from lot to lot.

   a. Enter the lower limit OD reading in the first box.

   b. Select the Control from the drop-down list.

c. Enter the upper limit OD reading in the remaining box. In the example in Figure D-20, the Positive control OD must be between 0.5 and 1.5 for a valid result.

Figure D-20 Control vs. RTV (OD)

d. Click OK when complete and then reselect the option to add criteria for other Control types if needed. The OD range can be edited during sample run programming when saving to an instrument.

5. **Control vs. RTV (Units)** defines Run Time Variables (RTV) for Controls based on the calculated result.

   a. Enter the lower limit value in the first box.

   b. Select the Control from the drop-down list.

   c. Enter the upper limit value in the remaining box. In the example in Figure D-21, the Positive control value must be between 1.5 and 8.0 for a valid result.

Figure D-21 Control vs. RTV (Units)

   d. Click OK when complete and then reselect the option to add criteria for other Control types if needed. The value range can be edited during sample run programming when saving to an instrument.

6. **Mean Rule** defines an OD range for the selected Control and defines the number of replicates required with a percent deviation from the mean. The mean is the average value of the control replicates.

   a. Enter the upper limit value in the first box.

   b. Select either > or >= from the first drop-down list.

   c. Select the Control from the second drop-down list.

   d. Select either > or >= from the third drop-down list.

   e. Enter the lower limit value in the next box.

   f. Enter the value (%) for the allowed distance from the mean in the designated box.

g. Enter the number of control replicates in the "Required number of valid controls" box. In the example in Figure D-22, all 3 replicates of the Positive control OD must be between 0.5 and 1.5. The mean will be calculated and all replicates must be within 15% of the mean.

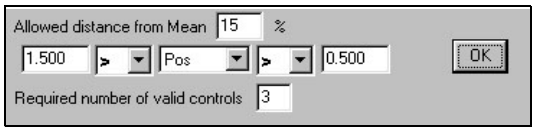

Figure D-22 Mean Rule

h. Click OK when complete and then reselect the option to add criteria for other Control types if needed.

7. **Median Rule** allows the programmer to set an OD range for the selected Control and then define the number of replicates required with a percent deviation from the median. The median is the middle replicate value. For the values 1.0, 1.3 and 1.4 the Median would be 1.3.

   a. Enter the upper limit value in the first box.
   b. Select either > or >= from the first drop-down list.
   c. Select the Control from the second drop-down list.
   d. Select either > or >= from the third drop-down list.
   e. Enter the lower limit value in the next box.
   f. Enter the value (%) for the allowed distance from the median in the designated box.
   g. Enter the number of control replicates in the "Required number of valid controls" box. In the example in Figure D-23, all 3 replicates of the Positive control OD must be between 0.5 and 1.5. All replicates must be within 15% of the median value.

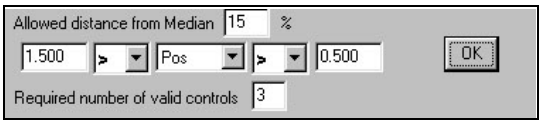

Figure D-23 Median Rule

   h. Click OK when complete and then reselect the option to add criteria for other Control types if needed.

D.4.4 Scaling Factor

Select the box "Remind User to Enter Scaling Factor" if the scaling factor for calculations will change from lot to lot.

A warning message will appear during programming of a sample run when saving to an instrument if the scaling factor is not changed from the default setting.

D.5 Messages

Select the Messages menu from the menu bar. This menu defines the criteria for interpreting patient sample results. The equations and curve fits defined in Validations will determine the options available for result interpretation. If a cutoff option was defined, only the Using Cutoff option will be listed (Figure D-24).

There are four menu options for defining messages: Using OD, Using Controls, Using Units, and Using Cutoff. Only one of these four options can be selected and defined.

Figure D-24 Messages, Cutoff Option

Figure D-25 Messages, Remaining Options

Warning: *It is in the programmer's best interest to write out the list of messages before defining the criteria in the software as there is no way to edit an interpretation once it has been defined. If a mistake is made, the message must be deleted and redefined. This will place it at the end of the list. If the message to edit is in the middle of the list, all messages must be deleted and redefined.*

Program the messages in ascending order, starting with an interpretation for a Negative result and working up to a Positive result. In the example in Figure D-26, a sample is considered Negative with a value between 0 and 25, Borderline from 25 to 30, Low Positive from 30 to 60, Positive from 60 to 200 and Strong Positive if greater than or equal to 200.

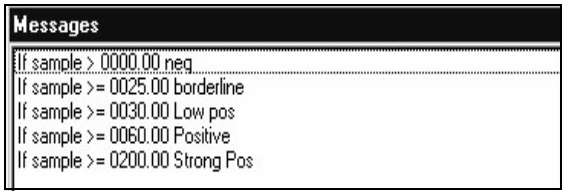

Figure D-26 Sample Result Interpretations

D.5.1 Using Cutoff

1. Select an option from the drop-down list next to Sample. The only options are > and >=.

2. Enter a value in the next box.

3. Select a math option from the next drop-down list. The options are +, -, * and /.

4. Enter the interpretation name in the last box; this is a free text box.

5. To define a Negative result, enter 0 and then select (*) for the option.

6. In the example in Figure D-27, a sample that is greater than or equal to 0 plus the Cutoff will be flagged as Positive on the report. In this case any value equal to the cutoff value will be considered a Positive result.

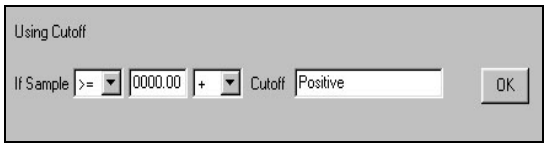

Figure D-27 Messages, Using Cutoff

7. Click OK and then define the next interpretation. If there are no additional interpretations, close the message window by clicking the X to return to the Main Window.

D.5.2 Using OD or Units

Allows the programmer to use an OD or unit value as a reference point for sample interpretation.

1. Select an option from the drop-down list next to Sample. The only options are > and >=.

2. Enter a value in the next box.

3. Enter the interpretation name in the last box; this is a free text box.

4. In the example in Figure D-28, samples with an OD greater than or equal to 0.40 will be flagged as Positive on the report.

   In the example in Figure D-29, samples with a final result greater than 1.0 will be flagged as Positive on the report.

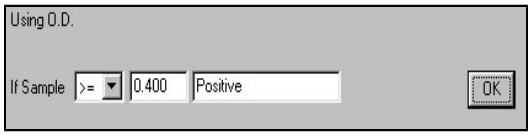

Figure D-28 Messages, Using OD

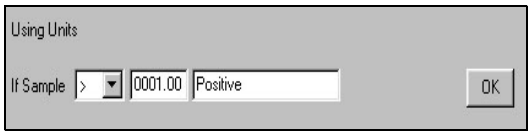

Figure D-29 Messages, Using Units

5. Click OK and then define the next interpretation. If there are no additional interpretations, close the message window by clicking the X to return to the Main Window.

D.5.3 Using Controls

Allows the programmer to use a defined Control as a reference point for a sample interpretation.

1. Select an option from the drop-down list next to Sample. The only options are > and >=.

2. Enter a value in the next box.

3. Select a math option from the next drop-down list. The options are +, -, * and /.

4. Select the Control to be used as the reference point from the last drop-down list. The list reflects only those control types previously defined.

5. Enter the interpretation in the last box; this is a free text box.

6. In the example in Figure D-30, samples with a result greater than 2 times the Calibrator will be flagged as Positive on the report.

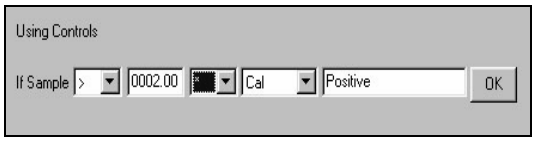

Figure D-30 Messages, Using Controls

7. Click OK and then define the next interpretation. If there are no additional interpretations, close the message window by clicking the X to return to the Main Window.

D.5.4 Other Messages Options

The remaining options in the Messages menu are for deleting interpretations or defining the color of the interpretation on the report (Figure D-31).

Figure D-31 Other Messages Options

"Delete" will delete only the interpretation that is selected. "Delete All" will delete all the interpretations.

Selecting an interpretation and then the option "Color" will open a window with a choice of colors to appear on the report.

D.6 Saving the New Assay File

1. Select File from the menu bar of the main Assay window.

2. Select Save As from the menu. The directory pathway will be C:\Program Files\Bio-Rad\PhD\EIA\ASA. Saving the file to any other directory will make the assay unavailable from the assay menu in the PhD software.

3. Enter the file name for the revised assay. The Bio-Rad naming conventions for a Bio-Rad assay file do not allow the use of spaces or special characters and are as follows:
   - Assay name_isotype (e.g., ASCA_IgG)
   - Assay name_isotype_single-point or multi-point (e.g., aCL_IgG_SP or aCL_IgG_MP)
   - Assay name_kit catalog number (e.g., dsDNA_96DS)

4. Click OK.

D.7 Modifying Drop-Down Lists in the Main Assay Window

Drop-down list options can be edited, added, or deleted for the following items.

Table D-1 Items with Editable Drop-Down Lists

PreDiluent (name)
Diluent (name)
Manufacturer
Wash Solution
Wash Step 1
Conjugate Add (name)
Wash Step 2
Substrate Add (name)
Stop Add (name)

NOTE: *Component names cannot exceed 19 characters.*

1. To add an option:
   - Right click the item's drop-down arrow, then click Edit.
   - The Modify Items window will appear (Figure D-33).
   - Click Insert, enter the new option, and click OK (Figure D-32).

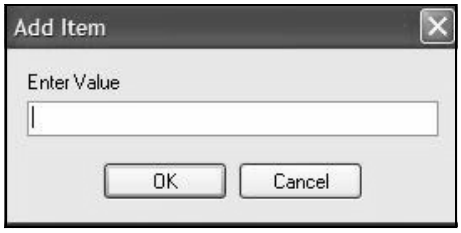

Figure D-32 Add Item Window

NOTE: *Users must not edit reagent names associated with Bio-Rad validated assay files.*

2. To edit an option:
   - Right click the item's drop-down arrow, then click Edit.
   - The Modify Items window will appear (Figure D-33).
   - Select the option to be edited.
   - Click Edit, enter the revised option, and click OK.

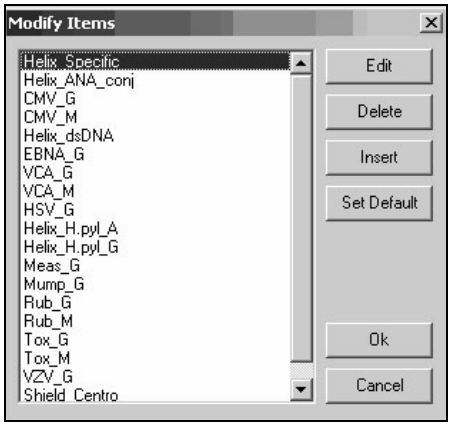

Figure D-33 Modify Items Window

NOTE: *Users must not delete reagent names associated with Bio-Rad validated assay files.*

3. To delete an option:
   - Right click the item's drop-down arrow, then click Edit.
   - The Modify Items window will appear.
   - Select the option to be deleted, then click Delete.
   - Click Yes on the Confirm window.

4. After adding, editing, or deleting an option, save the revised assay file:
   - Select File from the menu bar of the main Assay window.
   - Select Save As from the menu.
   - Enter the file name for the revised assay.
   - Click OK.

Appendix E: Full Upgrade of PhD Software and Bio-Rad Assay Files

The PhD EIA and IFA Software and Methods CD (Catalog No. 426-0246) includes executable files for installing additional assays validated by Bio-Rad and updates to currently validated assay files. Use the following procedure to install these assay files.

1. Verify that all connected workstations are turned off.

2. Turn on the computer.

3. Insert the PhD EIA and IFA Software and Methods CD into the CD-ROM drive of the PhD computer.

4. If the PhD Software Setup window does not appear, then right click the Start button at the bottom left of the screen and select Explore.

5. Select PhD in the CD-ROM drive. Double-click the file named PhD Setup.exe.

6. On the Welcome to the PhD Software Setup Wizard window, click Next (Figure E-1).

Figure E-1 PhD Software Setup Wizard Welcome Window

7. On the Choose Components window, select "EIA Install and Assay File Update" (Figure E-2).

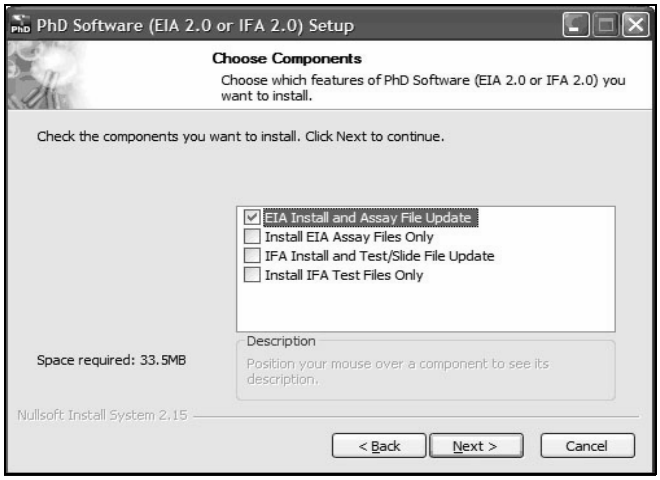

Figure E-2 Choose Components Window

NOTE: *To install EIA assay files only, without installing EIA software, select "Install EIA Assay Files Only".*

8. Click Next.

NOTE: *If only one assay file needs to be installed, select the applicable assay file, and then click Add.*

If more than one assay file needs to be installed, press and hold down the Ctrl key and select the applicable assay files, then click Add.

Updated assay files are preceded with a "+" symbol. If only updated assay files are to be installed, press and hold down the Ctrl key and select the assay files that are preceded with "+", then click Add.

9. The Assays Selection window has all available EIA assay files preselected (Figure E-3). If all EIA assay files need to be installed, click Add. All selected assay files will appear in the Assays to Install portion of the window (E-4).

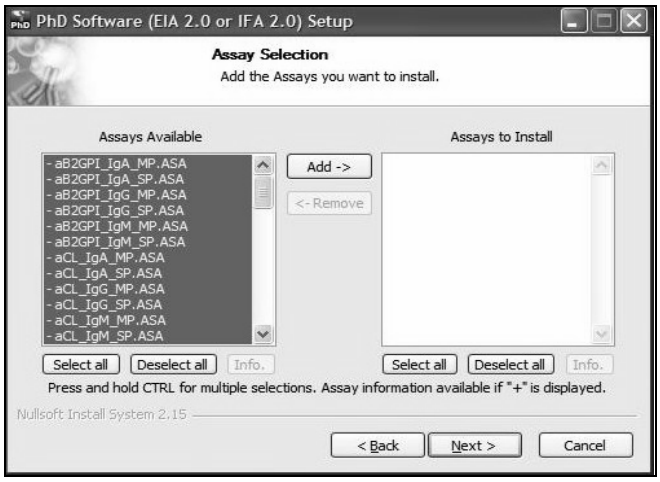

Figure E-3 Assay Selection Window with Available EIA Assay Files Preselected

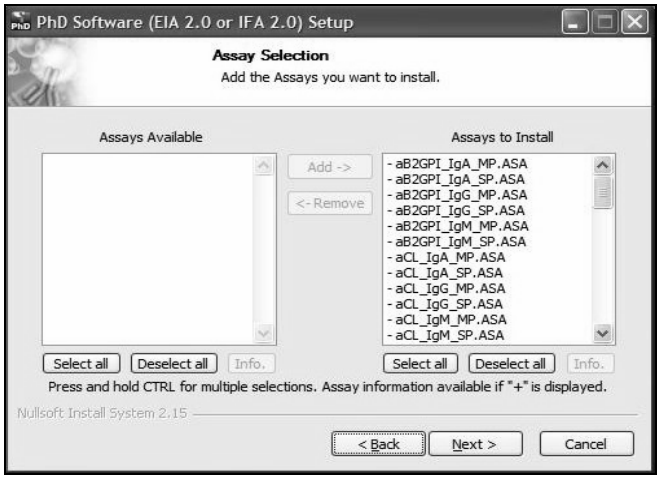

Figure E-4 Assay Selection Window with Confirmation of Assays to Install

NOTE: *To view additional information for an updated assay file, select the assay file preceded with "+", and then click the Info button. A text file window will be displayed that describes the updates to the selected assay file (Figure E-5).*

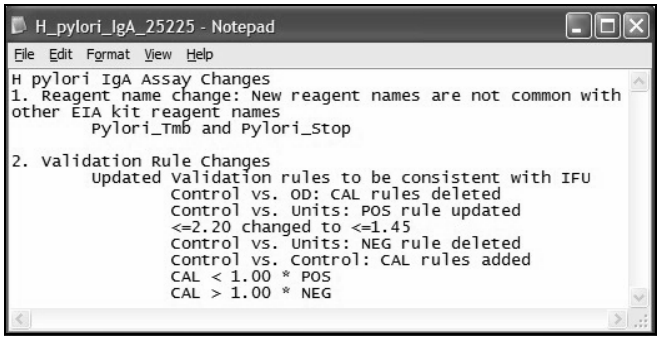

Figure E-5 Information Text File Window for Updated Assay File

10. Click Next.

NOTE: *The Restore EIA Assays window will be displayed to restore any assay files from the previous software installation that have not been updated with this installation. All existing files will be selected automatically. Click Restore to continue. To permanently remove older assay files, select the file(s) and click Remove.*

NOTE: *The initial software installation may require 10-15 minutes. Please be patient.*

11. On the Ready to Install/Update window, click Install (Figure E-6).

Figure E-6 Ready to Install/Update Window

12. On the Installation Complete window, click Next (Figure E-7).

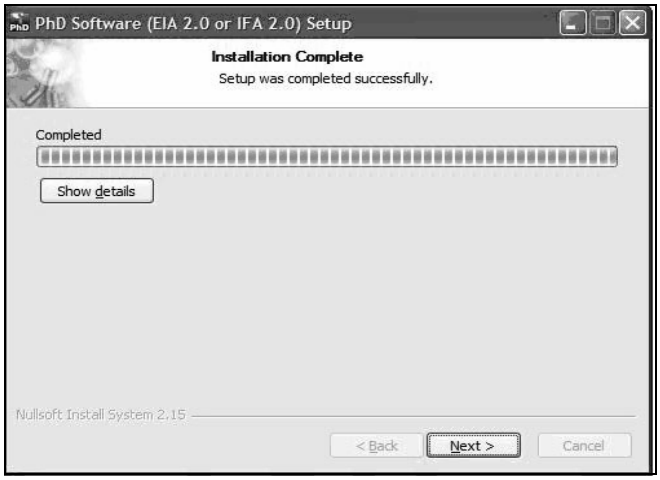

Figure E-7 Installation Complete Window

13. On the final window, click Finish (Figure E-8).

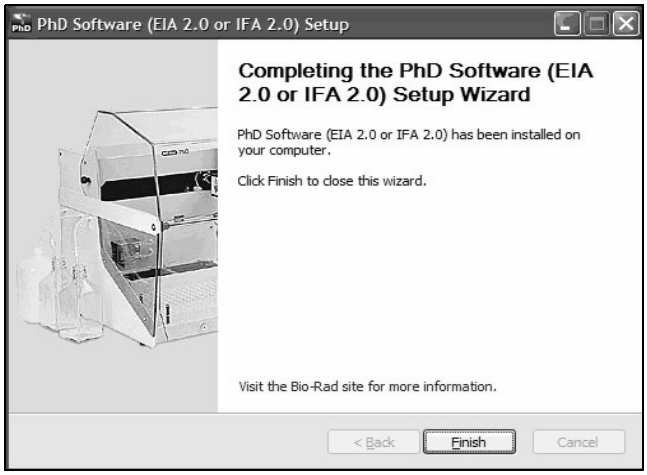

Figure E-8 PhD Software Setup Wizard Final Window

Appendix F: LIS Specifications

F.1 Introduction

The CLSI (formerly NCCLS) standard for information transfer is implemented in the PhD system software. Laboratory and information management systems (LIS) which correctly implement this standard will be able to receive PhD data with little or no configuration effort.

The ASTM standards are replaced by CLSI standards (identical to the ASTM standards).

The two implemented standards are:

- LIS1-A (low-level protocol) and
- LIS2-A2 (high-level protocol)

LIS1-A was formerly known as E-1381-95; LIS2-A2 was known as E-1394-97.

Connection to the LIS can be achieved:

- via a standard RS-232 serial port on the rear of the PhD computer, or
- via TCP/IP

Consult your LIS provider for information on configuring the appropriate RS-232 serial cable or TCP/IP connection for your LIS.

The following document describes how the CLSI protocols have been implemented in the PhD software. The document first discusses the High-Level Protocol, and then the Low-Level Protocol.

F.2 CLSI High-Level Protocols (LIS2-A2)

The following describes which ASTM record types and fields are supported by the PhD software.

The ASTM record types are:

- Header
- Patient Information
- Request Information
- Test Order
- Result
- Message Terminator

F.3 PhD LIS Options

F.3.1 PhD LIS Transmission

The PhD has two basic transmission options (Results and Worklist), and two formats (Patient-based and Test-based).

The Patient-based and Test-based formats differ in the arrangement of records, which is illustrated below. The Order records are omitted from this list to make it easier to understand.

Patient-based transmissions have the following format:

--All Controls for Test 1
--Patient(1) results for Test 1 **and any other Test results for Patient (1)**
--Patient(2) results for Test 1 **and any other Test results for Patient (2)**
.....
--All Controls for Test 2
--Patient(M) results for Test 2 **if this patient had no results on Test 1**
.....
--All Controls for Test 3
--Patient(N) results for Test 3 **if this patient had no results on Test 1 or Test 2**
.....

Test-based transmissions have the following format:

--All Controls for Test 1
--Patient(1) results for Test 1
--Patient(2) results for Test 1
.....
--All Controls for Test 2
--Patient (1) results for Test 2
--Patient (2) results for Test 2
.....

The actual content of the records is the same in both formats.

The PhD also has two output options:

- Send the Results to the LIS System (Menu item Results >> LIS)
- Send the Worklist to the LIS System (Menu item Worklist >> LIS)

When selecting the Results output option, all records (Patients, Controls, Blanks, Positive, Negative) are sent to the LIS receiver.

The output of "Results>>LIS" and "Worklist>>LIS" are otherwise identical, except that with "Worklist>>LIS" **no Result records are sent** (only the Header, Order, Patient Information, and Message Terminator records are sent).

F.3.2 PhD LIS Query

The EIA and IFA software can query the LIS for patient information. When starting the LIS query, the software will send every entered patient ID to the LIS. For every patient, the following data flow occurs (the example patient ID is 43210):

Data flow from EIA/IFA software to LIS:

Header Record:

H|\^&|||PhD_IFA^2^0^0^1||||||P|LIS2-A2|20060109112432

Query Record:

Q|1|43210||||||||||||O

Message Terminator Record:

L|1|N

Data flow from LIS to EIA/IFA software:

Header Record:

H|\^&|||||||||P|LIS2-A2|20060109112435

Patient Information Record:

P|1||43210||name^surname^suffix

Order Record:

O|1|43210||^^^ANA,20,40,80|R

Message Terminator Record:

L|1|N

There can be multiple Order records per patient; this is when there is more than one test that needs to be performed.

For IFA, the test name is followed by the dilutions.

In this example the test name is "ANA" and the dilutions are 1/20, 1/40, 1/80.

For EIA there is just the test name without dilutions:

O | 1 | 43210 | | | ^^^ANA | R

The above flow is for one patient and is repeated until all entered patients are processed.

F.4 CLSI Record Types

NOTE: *Underlined text indicates the fields that are supported by PhD software.*

Message Header Record (14 Fields):

Record Type ID (**H**) | Delimiter Definition (\^&|) | Message Control ID / Access Password | Sender Name or ID | Sender Street Address | Reserved Field | Sender Telephone Number | Characteristics of Sender / *Receiver ID* / *Comment or Special Instructions* / Processing ID | Version No. | Date and Time of Message <CR>

Sender Name or ID - PhD_EIA^2^0^0^15, is the software name and version number.

Processing ID = P indicates that the message will be processed as Production.

Version Number is the version level of the CLSI Specification, LIS2-A2.

Date and Time of Message is the date/time when the LIS transmission file is created by the PhD software (i.e., when the user selects Worklist>>LIS or Results>>LIS).

Example:

H | \^& | | | PhD_EIA^2^0^0^15 | | | | | | | P | LIS2-A2 | 20060503143647

Request Information Record (13 Fields):

Record Type (**Q**) | Sequence Number (1,2,3,…) | Starting Range ID Number | Ending Range ID Number | Universal Test ID | Nature of request Time Limits | Beginning Request Results Date and Time | Ending Request Results Date and Time | Requesting Physician Name | Requesting Physician Telephone Number | User Field Number 1 | User Field Number 2 | Request Information Status <CR>

The PhD software builds the Q record as:

Q | 1 | ^43210^ | | ALL

Patient Information Record (35 Fields):

Record Type (**P**) | Sequence Number (1,2,3,…) | Practice Assigned Patient ID | Laboratory Assigned Patient ID | Patient ID No. 3 | Patient Name | Mother's Maiden Name | Birth Date | Patient Sex | Patient Race-Ethnic Origin | Patient Address | Reserved Field | Patient Telephone Number | Attending Physician ID | Special Field 1 | Special Field 2 | Patient Height | Patient Weight | Patient's Known or Suspected Diagnosis | Patient Active Medications | Patient's Diet | Practice Field No. 1 | Practice Field No. 2 | Admission and Discharge Dates | Admission Status | Location | Nature of Alternative Diagnostic | Alternative Diagnostic Code and Classification | Patient Religion | Marital Status | Isolation Status | Language | Hospital Service | Hospital Institution | Dosage Category <CR>

The PhD software uses only the first five fields and builds the P record as:

<Number>**P** | <SampleCounter>… , where the sample counter counts all P records in the transmission.

Example:

P | 1 | | | | ^^^^

P | 2 | | | | ^^^^

Test Order Record (31 Fields):

Record Type (**O**) | Sequence Number | Specimen ID | Instrument Specimen ID | Universal Test ID | Priority | Requested/Ordered Date and Time | Specimen Collection Date and Time | Collection End Time | Collection Volume | Collector ID | Action Code | Danger Code | Relevant Clinical Information | Date/Time Specimen Received | Specimen Descriptor (Specimen Type ^ Specimen Source) | Ordering Physician | Physician's Telephone Number | User Field No. 1 | User Field No. 2 | Laboratory Field No. 1 | Laboratory Field No. 2 | Date/Time Results Reported or Last Modified | Instrument Charge to Computer System | Instrument Section ID | Report Types | Reserved Field | Location or Ward of Specimen Collection | Nosocomial Infection Flag | Specimen Service | Specimen Institution <CR>

Note that when patients are run in duplicate, two order records are generated.

Sequence Number is sequentially incremented until a record of greater hierarchical significance is transmitted (usually another Patient Information record), and is then reset.

Specimen ID field is composed from three components: *Sample ID, Worklist name* and *Sample number in the worklist*.

Example:

Peter^LISTEST^2. The third component is not included for non-patients;

Example:

BLANK^LISTEST.

Universal Test ID field is composed (according to CLSI protocol - see 6.6.1) from four (4) parts. The first three parts *(Part 1 - Universal Test ID, Part 2 - Universal Test ID name and Part 3 - Universal Test ID type)* are unused by the current LIS protocol and should be skipped in the Order record. *Part 4 - Manufacturer's or Local Code* is the only used part. Therefore, if the "LIS Assay ID" in the PhD assay is "ANA", then the Universal Test ID field is "^^^ANA".

Action Code is equal to:

For Worklist >> LIS: X for Patients. Controls are not transmitted.

For Results >> LIS: Q for Controls. Blank for Patients.

Report Types field is equal to "F" for all orders, indicating "final results".

Examples:

For Worklist >> LIS:

O|1| Peter^LISTEST^2|||^^^ANA1|R||||||X|||||||||||||||F

For Results >> LIS:

Control:

O|1|BLANK^LISTEST|||^^^ANA1|R||||||Q|||||||||||||||F

Patient:

O|1|Peter^LISTEST^2|||^^^ANA1|R|||||||||||||||||||||F

Result Record (14 Fields):

Record Type (**R**) | Sequence Number | Universal Test ID | Data or Measurement Value | Units | Reference Ranges | Result Abnormal Flags | Nature of Abnormality Testing | Result Status | Date of Change in Instrument Normative | Operator Identification | Date/Time Test Started | Date/Time Test Completed | Instrument Identification <CR>

According to CLSI protocol (see 6.6.1) when the Universal Test ID field is used in the Result record, there must be sufficient information to determine the relationship of the test results to the test.

There are two types of result records, one for numerical results (QUANT) and one for qualitative results (QUAL). For Controls, only one quantitative record is transmitted. This includes a numerical result and units, for example:

R|1|^^^ANA^QUANT|1.00|ANA#|||F

For Patients, two records are always transmitted: quantitative and qualitative.

Sequence Number is sequentially incremented until a record of greater hierarchical significance is transmitted (usually another Patient Information record), and is then reset.

Therefore:

- If only one Results record is sent for the order, its sequence number is 1
- If two Results records are sent for an order (QUANT and QUAL), the sequence numbers are 1 and 2

Universal Test ID field - The first part of this field is the same as in the Order record. It is followed by additional components that distinguish the result type - quantitative or qualitative.

If the "LIS Assay ID" in the PhD assay is "ANA", then the Universal Test ID field is:

"^^^ANA^QUANT"	for quantitative (numerical) results.
"^^^ANA^QUAL^^^F"	for qualitative (text message) results.

Data or measurement value - This is the numerical result (in concentration or index value) or the text message itself, depending on the type of result record.

Qualitative results are usually the text defined in the Messages in the PhD assay definition. If no messages are defined, this field is blank.

Units - For quantitative records, the units are defined in the PhD assay definition.

For qualitative result records or if there are no units defined, the field is blank.

Results Status <ResStatus> will be "X" for skipped lines or "F" for others.

Additional values for the Data or measurement value:

Quantitative records:

For Patient Result Records: Data Value may be a calculated result or "INVALID" or "SKIPPED" for a failed or skipped test.

"INVALID" is based on the Validation Rules included in the PhD assay definition.

Quantitative records:

The Data Value can be a result message. So, <ResultMessage> may be a Patient result message such as "POSITIVE", or "NEGATIVE", as defined in the Messages in the PhD assay definition.

It may also be "SKIPPED" if the well was skipped during sample preparation on the PhD workstation.

It may be "INVALID" for a failed test, based on the Validation Rules included in the PhD assay definition.

Examples:

(One Control sample)

O|1|BLANK^LISTEST^||ANA1|||||||Q|||||||||||||||F
R|1|^^^ANA1^QUANT|0.00|ANA#||||F

(Two Patient samples)

O|1|Jeroen^LISTEST^1||ANA1|||||||||||||||||||||F
R|1|^^^ANA1^QUANT|0.14|ANA#||||F
R|2|^^^ANA1^QUAL^^^F|neg|||||F
O|1|Jeroen^LISTEST^1||ANA2|||||||||||||||||||||F
R|1|^^^ANA2^QUANT|INVALID|ANA#||||F
R|2|^^^ANA2^QUAL^^^F|INVALID|||||F

NOTE: *The PhD software supports all three Message Terminator fields.*

Message Terminator Record (3 fields):

Record Type ID (**L**) | Sequence Number | Termination Code

Termination Code = **N** in the PhD software.

Example:

L|1|N

F.5 CLSI Low-Level Protocols (LIS1-A)

The Low-Level Protocol basically provides a wrapper for the High-Level Protocol. The Low-Level Protocol includes special characters for communication functions such as:

- Start of Transmission <STX>
- End of Transmission <ETX>
- Check Sums (in hexadecimal format)
- Line delimiters <CR><LF>

Since these definitions and rules for the low-level protocol are provided in the CLSI documentation, this document will not discuss timeouts, error handling, or other transmission rules. Instead, a few examples are provided, which indicate how the low-level protocol affects the transmission contents.

<ENQ>

Header Record

<STX>1H|\^&|||PhD^^3|||||||P|E 1394-97|20020313144000<CR><ETX>15<CR><LF>

Test1, Controls

<STX>2P|1<CR><ETX>3F<CR><LF>

<STX>3O|1|BLANK^ABC123^||Test1|||||||Q|||||||||||||F<CR><ETX>BA<CR><LF>

<STX>4R|1|^^^Test1^QUANT|0.00|EU1||||F<CR><ETX>3B<CR><LF>

<STX>5P|2<CR><ETX>43<CR><LF>

<STX>6O|1|CAL^ABC123^||Test1|||||||Q|||||||||||||F<CR><ETX>25<CR><LF>

<STX>7R|1|^^^Test1^QUANT|1.00|EU1||||F<CR><ETX>3F<CR><LF>

<STX>0P|3<CR><ETX>3F<CR><LF>

<STX>1O|1|POS^ABC123^||Test1|||||||Q|||||||||||||F<CR><ETX>42<CR><LF>

<STX>2R|1|^^^Test1^QUANT|1.06|EU1||||F<CR><ETX>40<CR><LF>

<STX>3P|4<CR><ETX>43<CR><LF>

<STX>4O|1|POS^ABC123^||Test1|||||||Q|||||||||||||F<CR><ETX>45<CR><LF>

<STX>5R|1|^^^Test1^QUANT|1.04|EU1||||F<CR><ETX>41<CR><LF>

<STX>6P|5<CR><ETX>47<CR><LF>

<STX>7O|1|NEG^ABC123^||Test1|||||||Q|||||||||||||F<CR><ETX>30<CR><LF>

<STX>0R|1|^^^Test1^QUANT|3.32|EU1||||F<CR><ETX>3F<CR><LF>

Test1, Samples

<STX>1P|6<CR><ETX>43<CR><LF>

<STX>2O|1|Andrew^ABC123^1||Test1|||||||||||||||||||||F<CR><ETX>92<CR><LF>

<STX>3R|1|^^^Test1^QUANT|0.04|EU1||||F<CR><ETX>3E<CR><LF>

<STX>4R|2|^^^Test1^QUAL^^^F|neg|||||F<CR><ETX>F7<CR><LF>

<STX>5P|7<CR><ETX>48<CR><LF>

<STX>6O|1|Cheryl^ABC123^3||Test1|||||||||||||||||||||F<CR><ETX>9E<CR><LF>

<STX>7R|1|^^^Test1^QUANT|1.28|EU1||||F<CR><ETX>49<CR><LF>

<STX>0R|2|^^^Test1^QUAL^^^F|Positive|||||F<CR><ETX>0C<CR><LF>

<STX>1P|8<CR><ETX>45<CR><LF>

<STX>2O|1|David^ABC123^4||Test1|||||||||||||||||||||F<CR><ETX>1C<CR><LF>

<STX>3R|1|^^^Test1^QUANT|0.16|EU1||||F<CR><ETX>41<CR><LF>

<STX>4R|2|^^^Test1^QUAL^^^F|neg|||||F<CR><ETX>F7<CR><LF>

<STX>5P|9<CR><ETX>4A<CR><LF>

<STX>6O|1|Eduard^ABC123^5||Test1|||||||||||||||||||||F<CR><ETX>90<CR><LF>

<STX>7R|1|^^^Test1^QUANT|3.79|EU1||||F<CR><ETX>51<CR><LF>

<STX>0R|2|^^^Test1^QUAL^^^F|HighPos|||||F<CR><ETX>6B<CR><LF>

<STX>1P|10<CR><ETX>6E<CR><LF>

<STX>2O|1|Fred^ABC123^6||Test1|||||||||||||||||||||F<CR><ETX>B7<CR><LF>

<STX>3R|1|^^^Test1^QUANT|0.03|EU1||||F<CR><ETX>3D<CR><LF>

<STX>4R|2|^^^Test1^QUAL^^^F|neg|||||F<CR><ETX>F7<CR><LF>

Terminator

F.6 CLSI TCP/IP Communications

The TCP/IP layer of the EIA/IFA software transmits only the high-level CLSI frames, so it does **not** (as with RS-232) transmit the special flow control characters and checksums as described in Section F.5.

The PhD software has the option of "Frame Pooling"; this means that all the intermediate frames (starting with the Header frame to the last frame, including the Terminator frame) are pooled and sent as one TCP packet. This results in a much faster performance.

When this option is turned off (in LIS Settings screen), every frame is sent as a separate TCP Packet.

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