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UltraPlex™

SmartReader OPERATINGMANUAL

This manual describes the operation of
the Pronostics SmartReader used to analyse
UltraPlex™ assays.

Pronostics Ltd. is an ISO 13485:2003 certified company

www.pro-nostics.com

1 Introduction

This is the Operating Manual for the UltraPlex™ SmartReader. It describes the instrumentation, procedures and software used to measure samples and analyse the data from those samples. The system scans samples in a 96 well plate and then analyses data produced from the UltraCodes™ to return clinically relevant data as an output. This data is in the form of a positive, borderline or negative result for all assays, together with the appropriate numerical value for semi-quantitative and quantitative assays.

2 Equipment

The UltraPlex™ SmartReader system, version UPLX 3.002 is composed of the following components:

- Inverted fluorescent microscope
- Automated stage and Z-drive
- Solenoid shutters
- PCI/shutter controller
- CCD camera
- Computer running UltraPlex™ SmartDecode software

2.1 Software

The reader software is the SmartDecode program, which is located on the Desktop of the computer. When this program is opened, the following window will appear:



Figure 1. SmartDecode software window on start-up

The options available are:



- Run an Assay – scanning program to systematically read a sample plate, well by well, and output the relevant data
- Maintenance Menu – used for various maintenance procedures. This menu is password protected and provided for use by authorised Pronostics service engineers only.
- There is also an Audit menu option on the menu bar, which is currently disabled. When the software is provided for long-term use, this will allow the user to view Levey-Jennings plots of the various calibrator and control sample values.

3 Safety precautions

1. **The ultraviolet rays emitted by the mercury burner are harmful and should not be directly looked at with unprotected eyes.** Consequently, the burner should never be turned ON whilst the lamp housing is not mounted on the rear of the reader unit. Ensure the door is closed during counting to avoid exposure to UV light produced by the mercury lamp. The exposure risk, however, is minimal as the unit cover encloses the reader system and the burner is present in a housing at the back of the reader unit.
2. High pressure gas is sealed within the mercury burner. Use of the lamp beyond the recommended life expectancy may result in deformation of the glass tube, and potentially tube rupture. When disposing of used mercury burners, always follow national and local laws and guidelines.
3. Following turning the mercury burner off please wait at least 20 minutes before restarting the burner to allow for cooling down.
4. Before opening the lamp housing for replacing the burner etc., be sure to set the main switch on the power supply to “O” (OFF), unplug the power supply unit’s output connector from the power supply unit to the lamp housing and wait for 20 minutes until the burner has cooled down.
5. The top panel of the lamp housing becomes very hot during operation. To prevent fire hazard, do not block the ventilation through the top panel or tilt the lamp housing during use.
6. Operation of the UltraPlex™ SmartReader should be limited to those individuals who have been trained by Pronostics Ltd.

4 System preparation

At least 20 minutes before use the following procedure should be carried out:

1. Turn on the high-intensity light source at least 20 minutes before sample measurement.
2. Turn on the computer and the transmitted light power supply of the microscope.
3. The computer will automatically boot and load Windows XP. Your password for logging on will have been provided to you during training.



The following items should be checked.

4.1 Mercury burner

Ensure that the mercury burner has ignited after being switched on by checking that the **Burner On** light on the mercury lamp power supply is illuminated. If the burner has not ignited, turn the power supply off, wait 15 minutes and then turn on again. If the lamp has still not ignited, please contact Pronostics technical support.

Ensure the lifetime counter on the mercury lamp power supply has not exceeded 300 hours. A new burner must be installed after every 300 hours, and only by Pronostics technical support. Please contact Pronostics technical support when the lamp use has reached 250 hours to arrange for installation of a new lamp.

4.2 Transmitted light lamp

Ensure the transmitted light lamp is on by checking that there is light appearing just above the transmitted shutter (you may need to turn the illumination intensity up to confirm this).

5 Running an assay

5.1 Introduction

This option is used to enable the system to scan each well of a 96 well plate containing fluorescent UltraPlex™ UltraCodes to gather both microparticle and fluorescent response information to allow for sample analysis.

When you run an assay using the SmartDecode software, access is required to the .dat file generated by the SmartStation, which encodes the sample barcodes and other information. Some installations will have a direct connection between the SmartStation and SmartReader, whereas others will have them sited in different locations, and hence the two pieces of hardware will not be directly connected. The instructions below reflect these two options for the installation of the UltraPlex™ hardware.

5.2 Getting started

1. If the SmartStation and SmartReader are not connected together, retrieve the .dat file from the SmartStation on completion of the robotic run. To do this, open the "Shortcut to output" folder on the SmartStation desktop and copy the appropriately date and time-stamped .dat file to the USB flash drive. Next, place the USB flash drive into a USB port of the SmartReader PC.
2. Place the reading plate on the automated stage using the spring-loaded clip to hold the plate in place, and **ensuring that well A1 is at the back and left**.



3. Open the SmartDecode software by double-clicking on the SmartDecode icon on the desktop.
4. When the SmartDecode program is accessed, the stage will move to the 'home' position, the transmitted shutter will open and the camera will determine that light is available to the CCD.
5. Within the SmartDecode program window click the 'Run an Assay' button.

5.3 Optimising the transmitted light level

1. An image of the reading plate will appear (this is an image of the centre of well A1). Depending on the level of transmitted light, there will be one of three messages in the bottom right of the screen: 'Intensity is Optimal' (Figure 2), 'Too Dim – Please Increase Intensity' (Figure 3) or 'Too Bright – Please Decrease Intensity' (Figure 4). If there is no image visible, then refer to the troubleshooting guide (Section 6, page 12).



Figure 2. Image of reading plate (optimal light level)



Figure 3. Image of reading plate (below optimal light level - too dim)



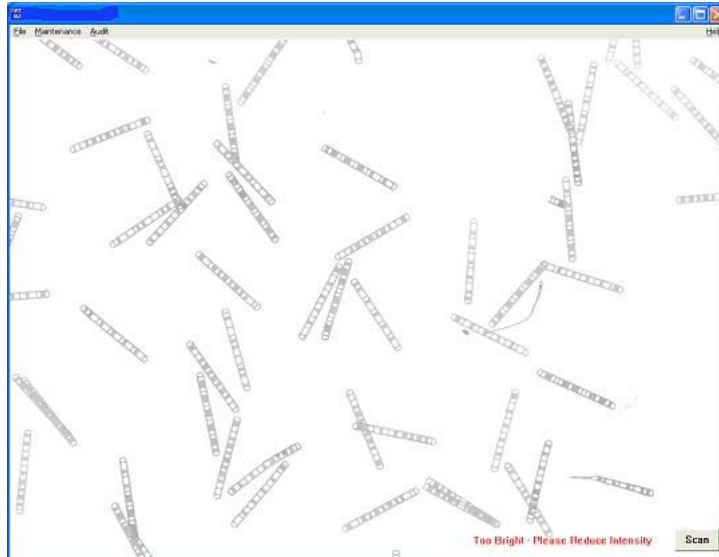


Figure 4. Image of reading plate (above optimal light level – too bright)

2. Focus the microparticles using the external focus device and then adjust the transmitted light level until an optimal setting is achieved (Figure 2). When this is achieved the door of the SmartReader should be closed.
3. Press the 'Scan' button (in bottom right corner of screen).

5.4 Select assay configuration file

1. A directory of files will appear (Figure 5). The location shown will be that which was last accessed, so typically you will be presented with a list of .dat files in the appropriate folder, whether they be on the E: drive (USB flash drive or other removable media device) or on the SmartStation PC (when SmartStation and SmartReader are directly connected).

If the correct location for the appropriate .dat file is not displayed, then navigate to the correct location using Windows. A shortcut to the SmartStation PC is located on the desktop of the SmartReader PC.

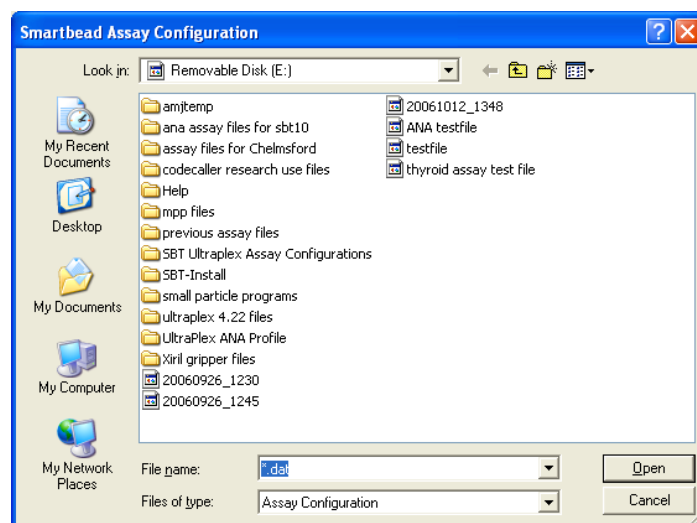


Figure 5. Removable media device directory window



2. Select the relevant SmartStation robot file ID (or “.dat” file) and press ‘Open’.

If the file has not been used previously, the system will begin scanning the reading plate. However, if the selected file has been used before, an error window will appear (Figure 6). If you are intentionally re-scanning a plate that has been run previously, press ‘Yes’ (the data will be placed into a new folder and the old data retained) and the system will begin scanning. If you press ‘No’ you will be returned to a live view of the UltraCode microparticles (Section 5.3, page 4).

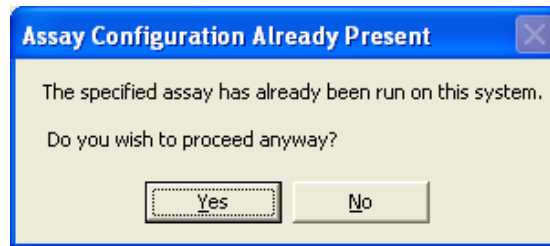


Figure 6. Error window dialog box

5.5 Starting scanning

Following selection of the SmartStation robot file the system will begin scanning the read plate. When it starts to do so, a ‘Scanning progress’ window will appear as shown in Figure 7.

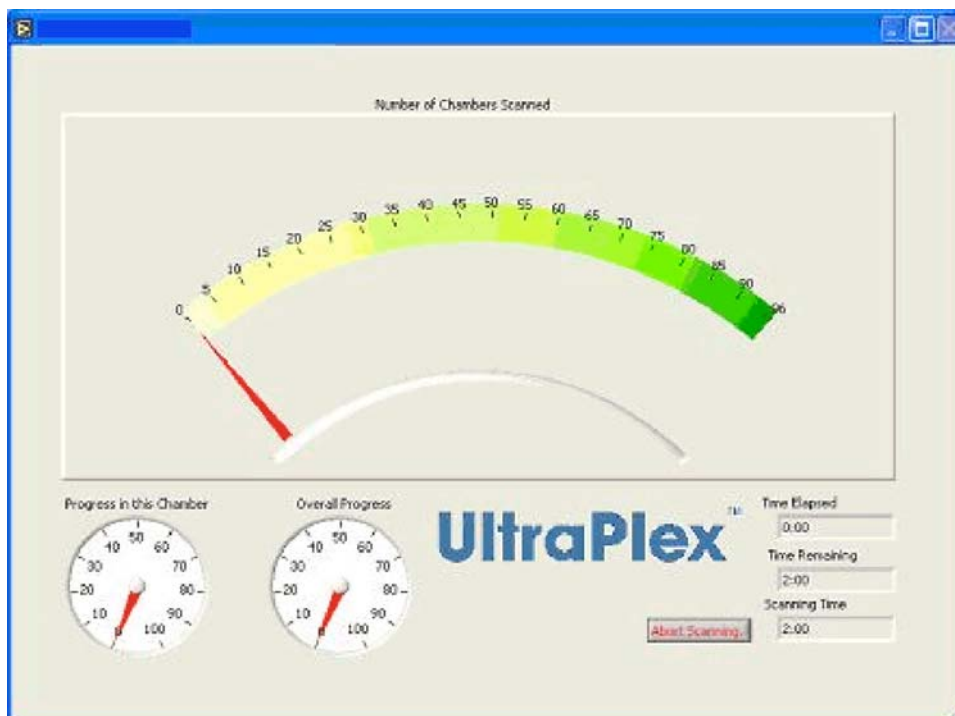


Figure 7. ‘Scanning progress’ window

This window shows the progress of the scanning of the reading plate. It shows the number of chambers scanned, the percentage of the current chamber scanned, the overall percentage scanned of the plate, the time elapsed (in an hours:minutes



format), the time remaining for scanning and total scanning time for the full plate (96 wells).

5.6 System check

At a number of points early on in the scanning process the system will determine that all the required components are on and functioning correctly. Some of these assessments are done during the counting of the first well, so it is important that the user remains with the system until the first well has completed counting to determine if there are any problems.

Please refer to the troubleshooting guide (Section 6, page 12) for help with any error messages.

5.7 Scanning progress and abortion of scanning

Throughout the scanning procedure the Scanning progress window remains open (Figure 8).

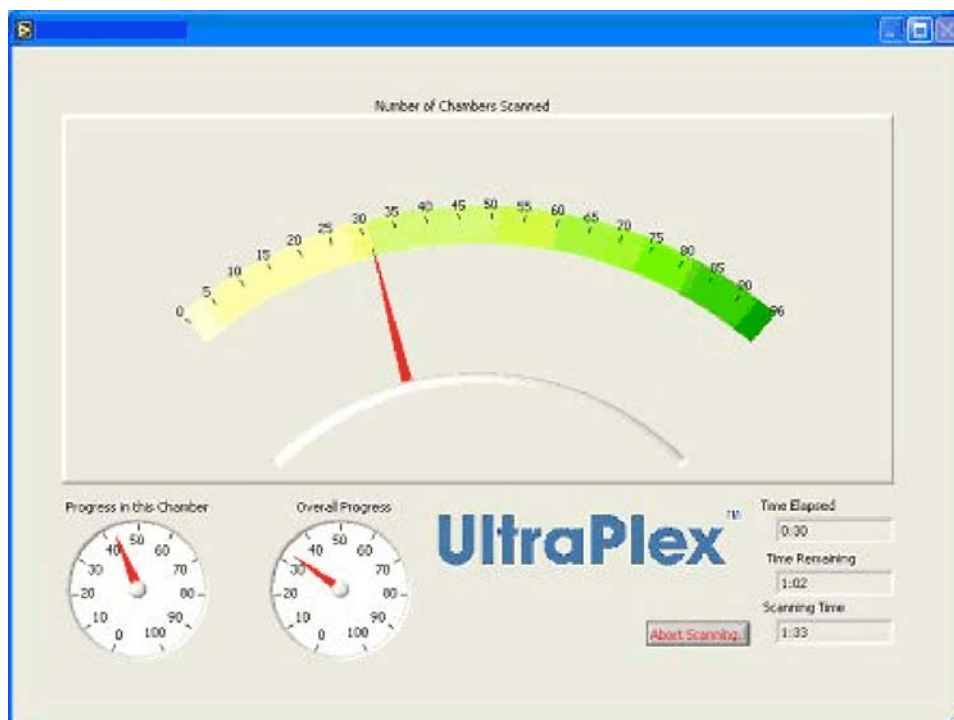


Figure 8. 'Scanning progress' window during counting

To stop scanning the plate at any time, press the 'Abort Scanning' button. You will be prompted to confirm the abort procedure (Figure 9).

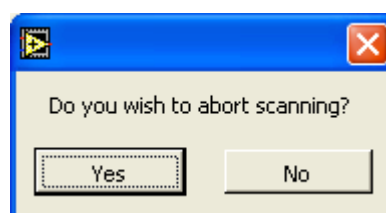


Figure 9. Abort scanning confirmation window



By aborting the scanning procedure the system will stop reading the plate at whatever point the 'Abort Scanning' button was pressed and return you to optimisation of the light intensity (Section 5.3, page 4). **Please note that all data collected so far will be lost.**

Depending on the point at which the 'Abort Scanning' button was pressed, it may take a few minutes to return to the start position. If you wish to abort the assay entirely, then simply close the SmartDecode program.

5.8 Completion of scanning

When the full reading plate has been scanned, the standard curves for any quantitative assays will be displayed on screen one at a time (Figure 10).

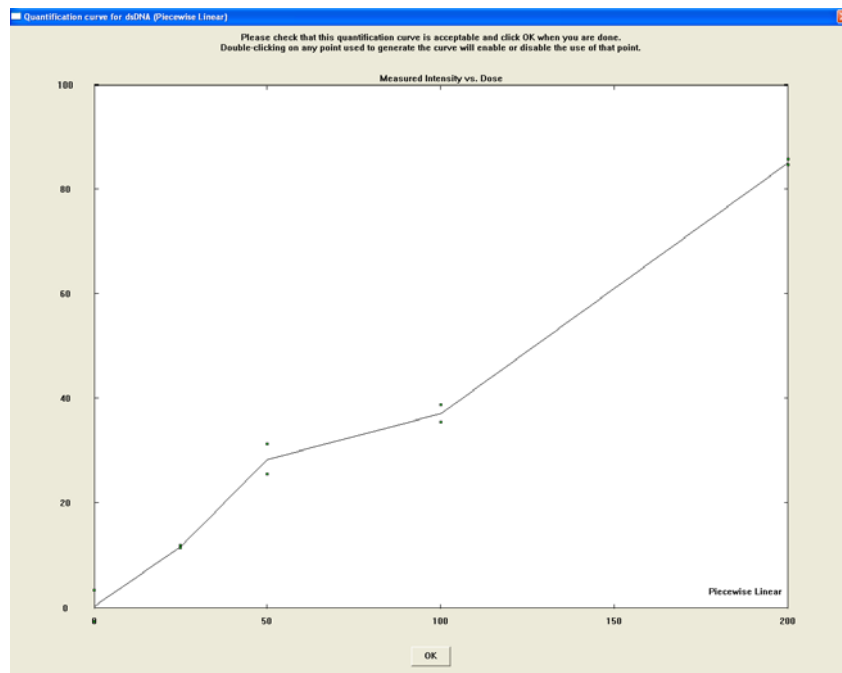


Figure 10. Standard curve (dsDNA example)

The replicate points are plotted on the graph as green squares. If the curve has a missing dose or outlying replicate, these will still be shown on the graph.

If the standard curve is acceptable, please press 'OK'. If one of the points on the curve has a bad replicate, then it can be discarded. Double-click on the green square of the replicate to be discarded and the curve will be redrawn (Figure 11). The suppressed data point will turn into a red square. Any point can be suppressed or once again taken into account by double-clicking the data point. The instructions for suppression of data points are shown at the top of the graph window.



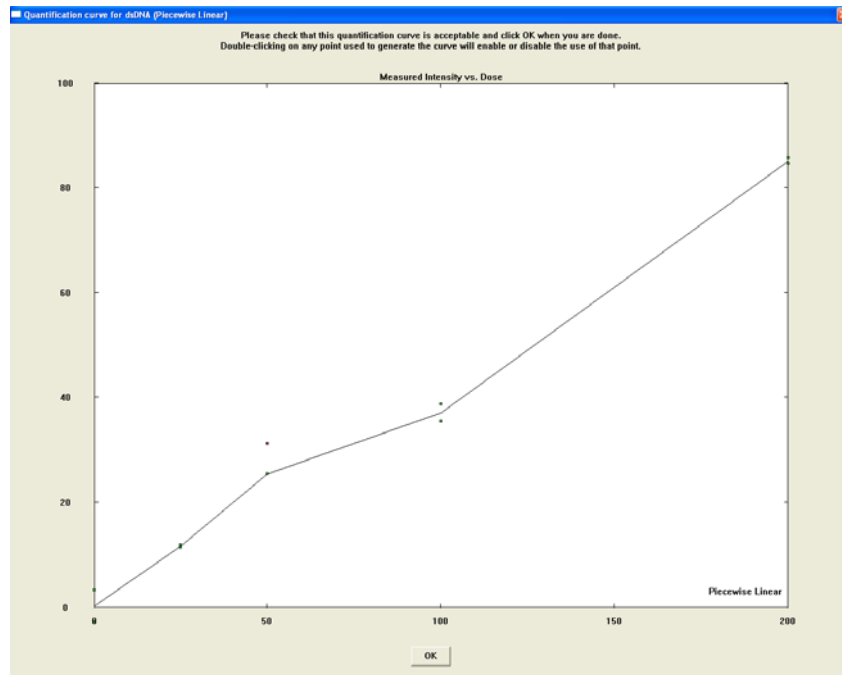


Figure 11. Modified standard curve (dsDNA example)

When you press 'OK' to confirm that the standard curve is suitable for analysis, details of the control samples in the assay may appear in the results window. This screen will not necessarily be shown for all assay types.

If displayed, the control sample results should be compared against the expected control sample results from the kit insert. If the results match, then the assay has performed as expected.

Depending on the assay that has been run, you may now have up to four options:

- Press the 'Print' button to print a hard copy of the standard curves (see Section 5.9).
- Press the 'LIMS' button to transmit the data to a LIMS host via an RS232 connection.
- Press the 'Export' button to add various statistical parameters to the output file (recommended for advanced users only).
- Press the 'Exit' button to exit the system.

5.9 Printed results

When you select **Print** from the results window a dialogue box will open and ask you for details of the printer, etc. Press 'OK' and you will be provided with a print-out containing some of the data from the assay run. The exact details of what is printed will depend on the assay that has been run, but may include standard curves (if any), control samples and/or sample data.

An example of page 1 of this print-out is shown in Figure 12.



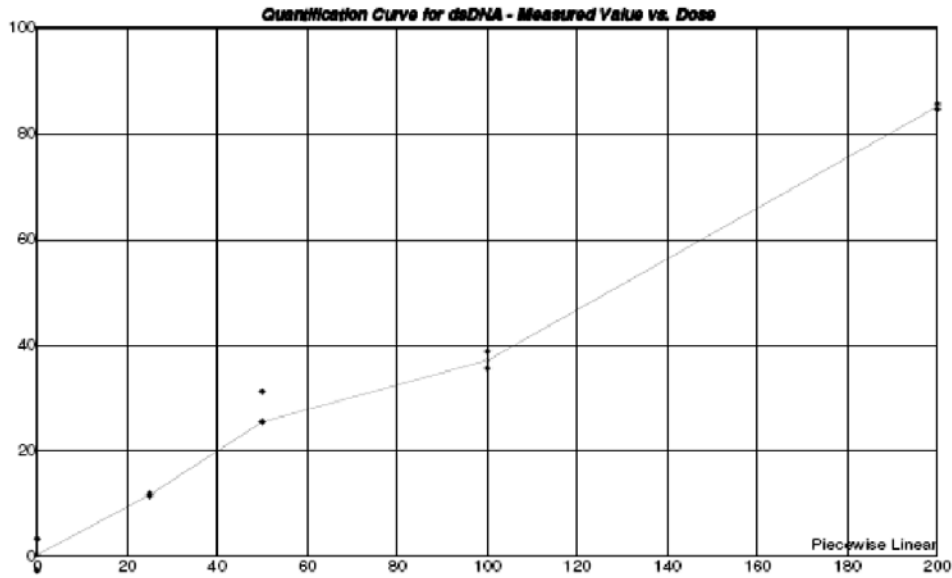


Figure 12. Standard curve (UltraPlex™ ANA)

In order to print the results from your samples, please navigate to the folder on the hard disk that contains your results file. This is located in:

C:\DocumentsandSettings\All Users\(\Shared) Documents\SBT UltraPlex Assay Results\

Folders are named by the date and time of the assay run. Within this folder is an Excel spreadsheet called **ANA_results.xls** (or Thyroid_results.xls etc., depending on the assay that has been run). This document contains a number of different worksheets, each with different information in it. The main results worksheet is called **results** and contains the data from all of the samples in an easy-to-read format (Figure 13). More detailed results can be found in the other worksheets of this **results.xls** file, including the number of microparticles analysed for each assay in each well and raw fluorescence intensities.

Samples	Qmax zero	cent B	SSA	SSB	Sci-70	Jo-1	Sm	SmRNP	U1 snRNP	dsDNA	
2648	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
2649	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
2681	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
2682	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
2683	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
2687	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
2702	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
06H188878	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5734	Normal	Positive	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5735	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5736	High	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	56	Positive
5733	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	12	Neg
06H188890	Normal	Positive	Neg	Neg	Neg	Neg	Borderline	Neg	Neg	36	Neg
06H188886	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5737	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	23	Neg
06H188881	Normal	Neg	Neg	Neg	Positive	Neg	Neg	Neg	Neg	< 10	Neg
5738	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5739	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5740	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5741	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5742	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5743	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Borderline	Neg	< 10	Neg
5744	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5745	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5746	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5747	Normal	Neg	Neg	Neg	Positive	Neg	Neg	Neg	Neg	13	Neg
5748	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5749	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5750	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	43	Borderline
5751	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5752	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg
5753	Normal	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	< 10	Neg

Figure 13. Sample data (UltraPlex™ ANA)



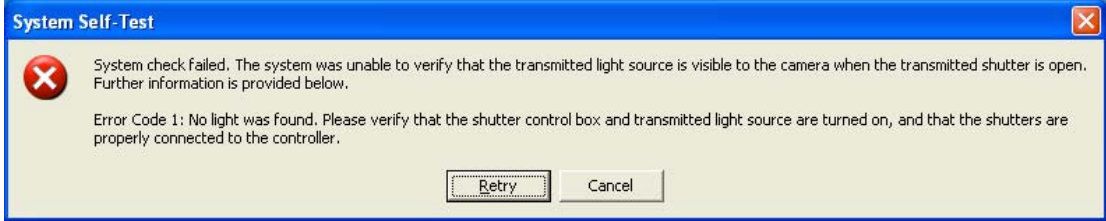
5.10 Data storage

The data in the Excel data sheets is stored on the Hard Disk of the computer running the SmartReader. The path to the data is shown in Section 5.9. This data is therefore accessible in future days and weeks, as it is not deleted automatically by the SmartDecode software. **However, we should remind all users that responsibility for backing up this data is theirs, and that a suitable regimen for ensuring data integrity should be instigated.**



6 Appendix A. Troubleshooting guide

6.1 Error message upon opening the SmartDecode software



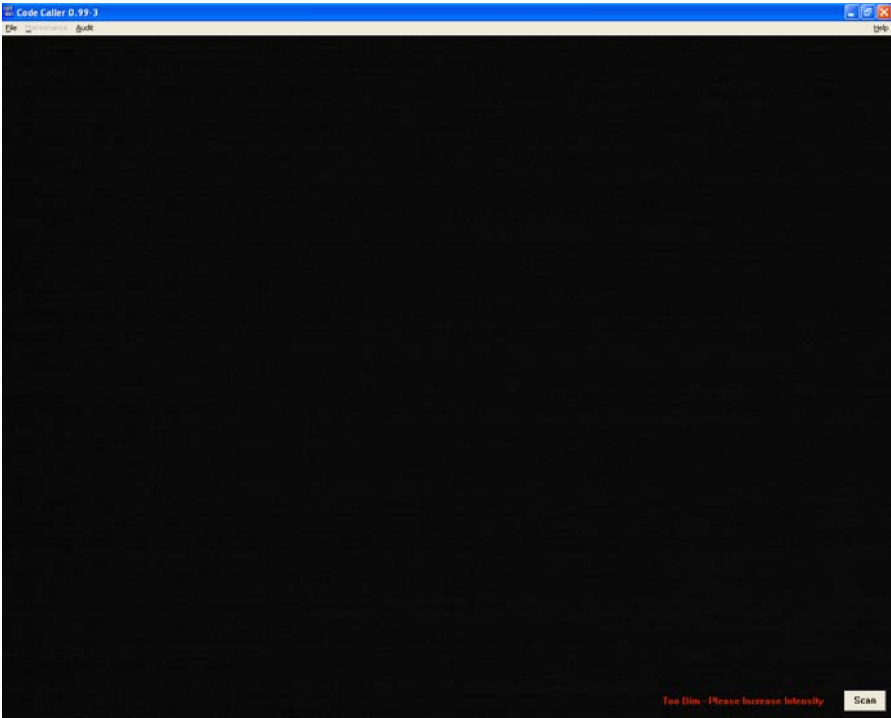
This message indicates that the transmitted light (necessary for decoding the microparticles) is not available to the camera.

Reason	Fix
Transmitted light failure	Confirm transmitted light power source switched on and dimmer switch not turned down very low.
Stage co-ordinate problem	Perform stage co-ordinate calibration (see Appendix B, page 14).
Camera problem	Ensure the camera cable is connected to the camera.

Having checked all the above components, press **Retry**. The system should function as normal.

If the problem persists, please call Pronostics technical support.

6.2 Error message upon pressing Run an Assay button 'Too Dim'



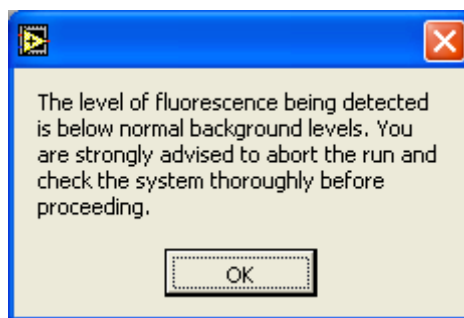
This message indicates that the transmitted light (necessary for decoding the UltraCode microparticles) is not available to the camera.

Reason	Fix
Transmitted light failure	Confirm transmitted light power source switched on.
Stage co-ordinate problem	Perform stage co-ordinate calibration (see Appendix B, p. 14).
Camera problem	Ensure the camera cable is connected to the camera.

Having checked all the above components, close this window and open the SmartDecode software again and **Retry**. The system should function as normal.

If the problem persists, please call Pronostics technical support.

6.3 Error message after scanning of the first well is complete (1)

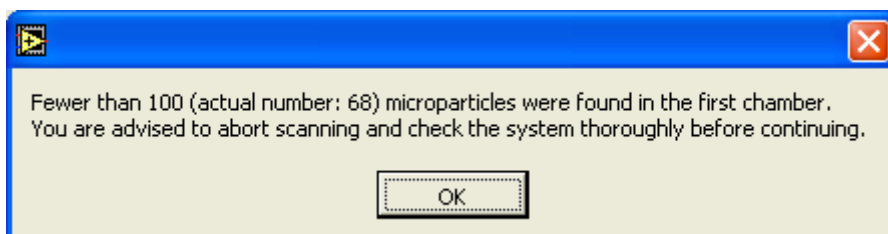


This indicates that the level of fluorescent signal being detected is too low.

Reason	Fix
Burner is not lit	Check that the 'Burner On' light on the mercury lamp power supply is illuminated. If not, press the 'Abort Scanning' button and exit the SmartDecode software, turn the power supply off, wait 15 minutes and then turn 'On' again and check that the burner has ignited.
Burner is lit, but low fluorescence levels detected	Please contact Pronostics technical support.

If the burner still does not ignite, please call Pronostics technical support.

6.4 Error message after scanning of the first well is complete (2)



This message indicates that the number of microparticles counted is low.



Reason	Fix
Light level too dim or too bright	Abort the scanning process and exit the SmartDecode software. Open the SmartDecode software from the Desktop and 'Run an Assay' again. Ensure that the transmitted light level is in the 'Optimal' range.
Poor focus point at the beginning of scanning	Abort the scanning process and exit the SmartDecode software. Open the SmartDecode software from the Desktop and 'Run an Assay' again. Ensure that the microparticles are in focus.

Note: For the UltraPlex™ ANA 10plex assay the number of microparticles for optimal counting should be >200. For the UltraPlex™ thyroid 3-plex assay, the number should be >60 and for the UltraPlex™ Coeliac 4-plex assay, the number should be >80. If the number is below an average of 10 microparticles per code then follow the 'fix' process above. If the average number is higher than this ignore the error message and press OK to continue scanning.

7 Appendix B. Scanning stage co-ordinate check

If the stage is suspected of being mis-aligned (See troubleshooting guide, Section 6.2) please call Pronostics technical support, who will talk you through the re-alignment procedure.

8 Appendix C. User maintenance

Perform the following maintenance weekly:

- 1) Wipe down any surfaces at risk of contamination by samples and reagents using soft wipes and alcohol.

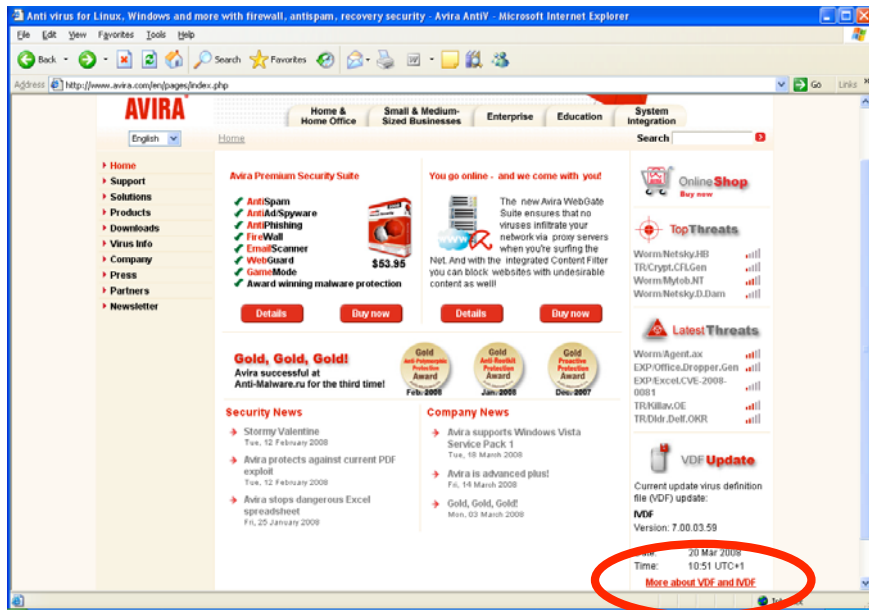
Pronostics Ltd and associated partners will perform periodic maintenance checks on systems at customer sites, with the interval pre-agreed and dependent on patient sample throughput.

8.1 Updating Avira anti-virus definitions

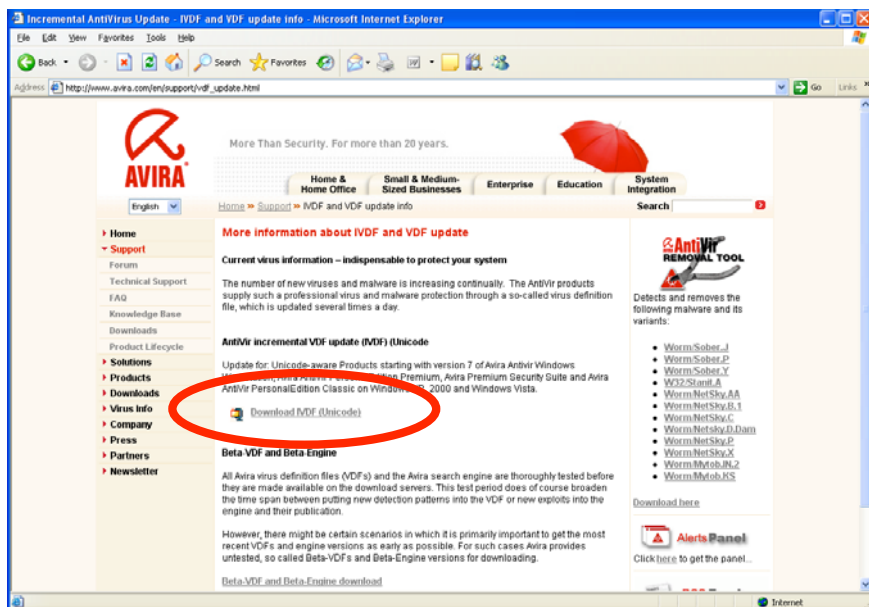
The SmartReader is provided with up-to-date Avira anti-virus software. However, after three days without the virus definition file being updated a warning will appear on the SmartReader. This will not impair its performance in any way, but the following instructions can be carried out to update the virus definitions file as required. We recommend that this is carried out regularly.

1. On any computer that is connected to the internet, go to www.avira.com.
2. Click on "more about VDF and IVDF" near the bottom right hand side of the web page.

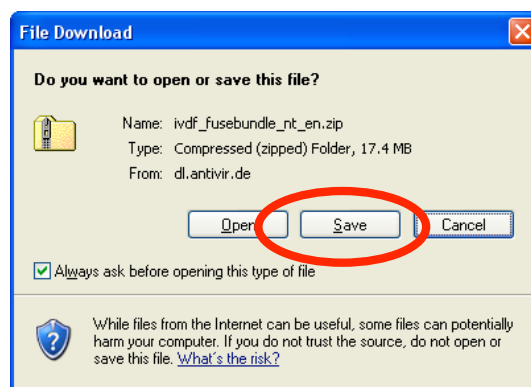




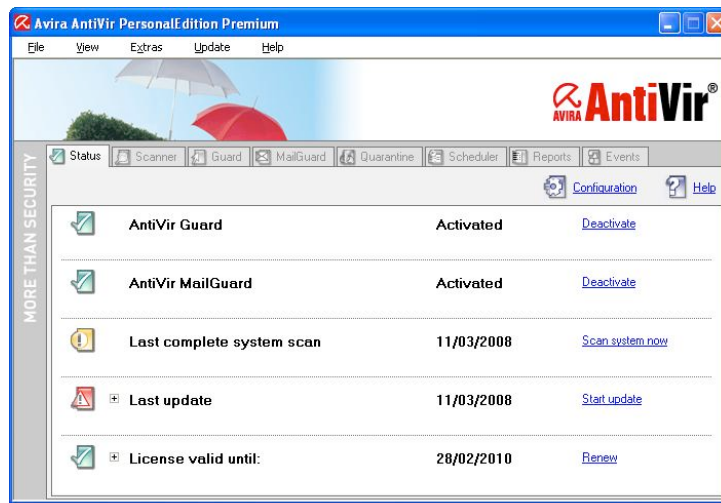
3. You will be taken to the information about IVDF and VDF update page



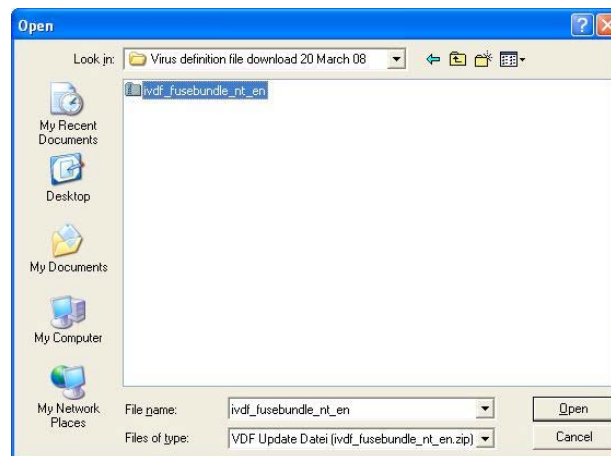
4. Click on "Download IVDF (Unicode)". This will open a window asking you to open or save the file "ivdf_fusebundle_nt_en.zip".



5. Click on "Save" and save the file to a USB flash drive or other portable memory.
6. Take this file to the SmartReader that requires an update to its virus definition file.
7. Open the Avira Personal Edition Premium software by double-clicking the red umbrella in the task bar on the bottom right hand side of the screen.



8. Click on "Update" from the menu bar at the top of the screen, then select "Manual update".
9. A box then appears asking for a VDF Update file. Navigate to the file on the USB flash drive that has just been downloaded from the Avira website, select it and then click "Open".



The virus definitions file will be updated to the most recent version. We recommended that the user then carries out a manual system scan to determine if any viruses are present on the system.



9 Appendix D. Contacts

For technical support or maintenance issues contact:

Pronostics Ltd.
Babraham Hall
Babraham
Cambridge
CB22 3AT
Telephone: +44 (0)1223 496 730
Email: support@pro-nostics.com

10 Appendix E. Operating conditions

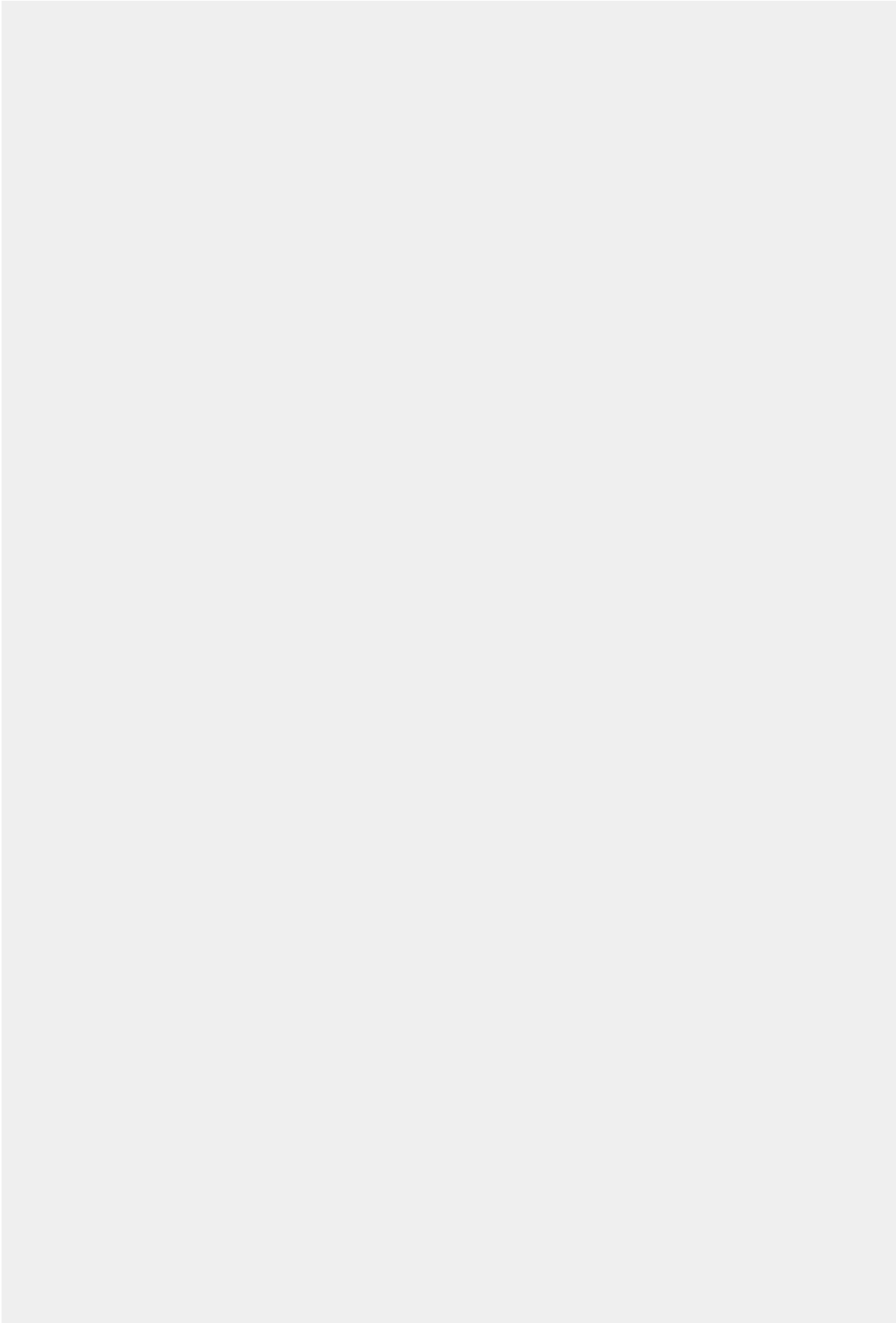
a.c. 50 Hz
220 to 240 volts
Rated value amps: 2.5 A
Temperature Range: 15 to 30°C

11 Appendix F. Installation requirements

Bench area: 1 square metre
Mains sockets: 1
Weight: 20Kg



12 Appendix G. Notes



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Pronostics Ltd. is an ISO 13485:2003 certified company

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