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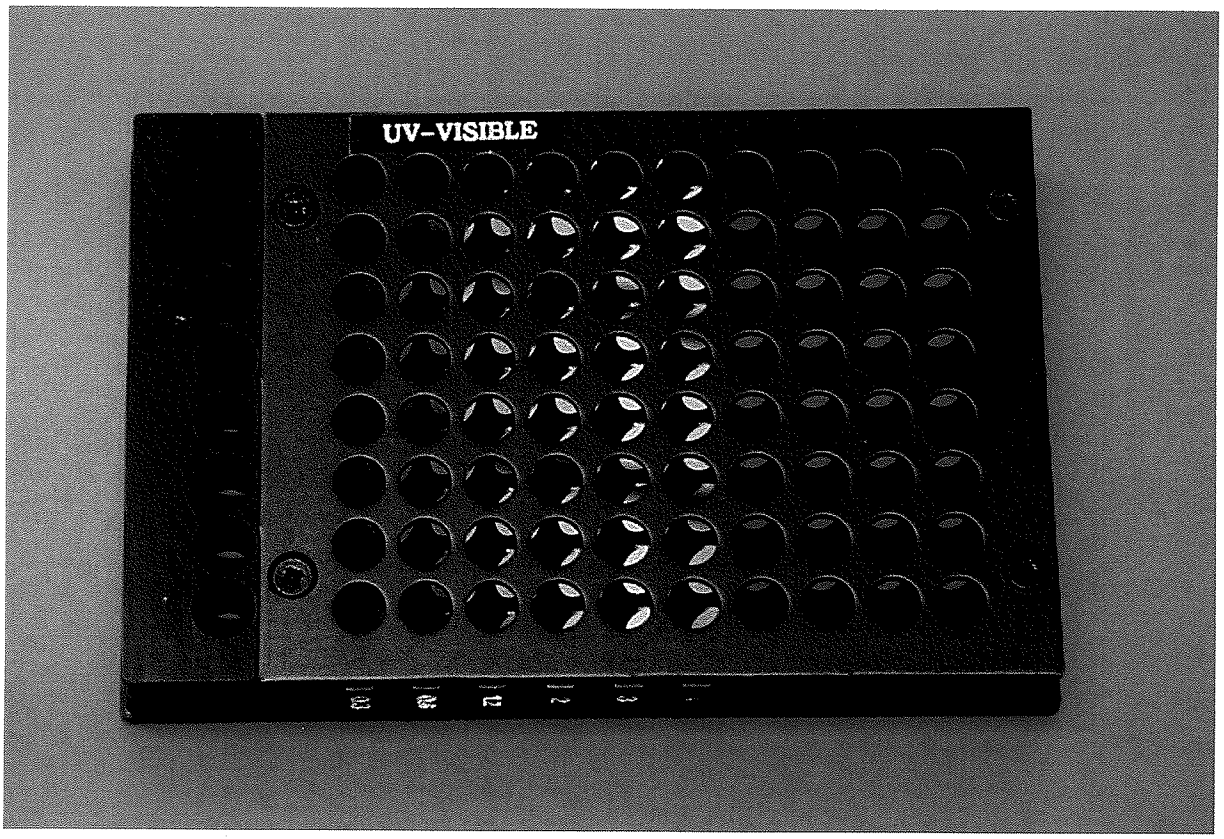
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Multiskan Verification Plate

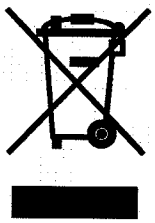
User Manual

User Manual Rev. 1.1; Dec. 2003, Cat. no. 1508470

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1. Introduction

The Multiskan Verification Plate is used to verify correct measurement performance of any of the following Thermo Electron microplate photometers:

1. Multiskan Ascent®
2. iEMS Reader MF
3. iEMS Analyzer [1,2]
4. Multiskan EX
5. Multiskan RC
6. Multiskan MS [1]
7. Multiskan MCC 340 MK II [1,2]
8. Multiskan Multisoft [1,2]
9. Multiskan Bichromatic [1,2]

The plate enables the user to verify photometric accuracy and precision at six absorbance levels covering an absorbance range of 0.3 to 2.0 Abs (*instruments 4 - 9*)/4.0 Abs (*instruments 1 - 3*) and at eight pre-selected wavelengths from the wavelength range of 340 to 750 nm (*instruments 4 - 9*)/850 nm (*instruments 1 - 3*). The items included in the Multiskan Verification Plate offer are as follows:

- NPL (National Physical Laboratory) traceable Verification Plate
- Certificate of Calibration for the Verification Plate
  Reference absorbance values for eight pre-selected wavelengths
- PVT Diskette
  Performance Verification Test (PVT) Sessions for Ascent Software®
  Reference absorbance files for eight pre-selected wavelengths
- Multiskan Verification Plate User Manual
- Carrying case for protecting the verification plate
- Order form for recalibration (re-certification)

1 Discontinued instrument 2 Manual verification

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1.1. Verification Plate and NDF glasses

The Verification Plate (*Fig. 1*) comprises six Neutral Density Filter (NDF) glasses with the following nominal absorbances: 0.3 Abs; 0.6 Abs; 1.2 Abs; 2.0 Abs; 3.0 Abs, and 4.0 Abs. The NDF glasses can be calibrated at any of the eight wavelengths ranging from 340 to 850 nm. Default wavelengths are 340 nm, 405 nm, 414 nm, 450 nm, 492 nm, 540 nm, 620 nm, and 690 nm.

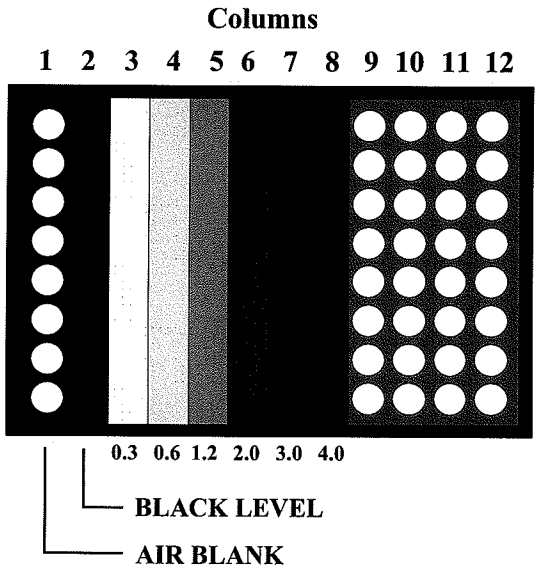


Fig. 1 Multiskan Verification Plate with six NDF glasses (0.3 Abs, 0.6 Abs, 1.2 Abs, 2.0 Abs, 3.0 Abs, and 4.0 Abs)

1.2. PVT Sessions for Ascent PC Software

The PVT Diskette contains the following Performance Verification Test (PVT) Sessions to be used with Ascent Software®:

- PVT Session for Multiskan Ascent®
- PVT Session for iEMS Reader MF
- PVT Session for Multiskan EX/RC/MS

The PVT Session verifies photometric accuracy and precision at six absorbance levels for one wavelength at a time. Each absorbance level is tested in eight microwell positions. After measurement, the test results are shown as spreadsheets, including raw measurement data, test calculations and the final test report with PASS/FAIL information.

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Note *It is strictly forbidden to sell, transfer or distribute to the public any copy of the Thermo Electron Ascent Software® or PVT Sessions. The Ascent Software® CD or PVT Diskette may be copied for backup purposes only.*

1.3. Reference Absorbance Files

The reference absorbance data is included in the Certificate of Calibration and can be found as reference absorbance files on the PVT Diskette. Each of the eight files are named *STDxxx.TXT,* where *xxx* corresponds to the wavelength at which the six NDF glasses are measured to obtain the reference absorbance values. These files are read-only.

1.4. Recalibration

It is recommended to recalibrate the Verification Plate first one year after the initial calibration and then every two years. You should contact Thermo's service department at least two months before the due date of your plate's calibration to obtain the return number (RGA) for the recalibration order form. The calibration due date is printed on the PVT Diskette and the Certificate of Calibration.

The recalibration process uses dedicated, traceable standards and instrumentation found at Thermo Electron. These standards are periodically and rigorously characterized by the National Physical Laboratory in the U.K. (NPL) to ensure the high quality of our services and of your results.

Note *The recalibration order form must contain the RGA return number, serial number of your verification plate and the relevant contact information. Failure to provide all necessary data will incur additional charges.*

2. Software and PVT Session Installation

2.1. Installation of the Ascent Software

Insert the Ascent Software® CD into your PC and installation starts automatically. Follow the instructions on your screen and answer any questions required.

The Ascent Software® runs under the Windows® operating system 3.1 or greater and the Ascent Software® version must be 2.4 or greater for the PVT Session. See more detailed requirements for the PC hardware from the *Ascent Software® User's Guide*.

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2.2. Installation of the PVT Session and reference absorbance files

The PVT Diskette contains the following PVT Session files – *always four per instrument* - and reference absorbance files (STDxxx.TXT) for the eight wavelengths specified.

PVT Session files	Corresponding instrument
PVTEST.SED, PVTEST.VTB, PVTEST.VTG, PVTEST.LAY	Multiskan Ascent
PVTEST**E**.SEE, PVTEST**E**.VTB, PVTEST**E**.VTG, PVTEST**E**.LAY	iEMS Reader MF
PVTEST**M**.SEE, PVTEST**M**.VTB, PVTEST**M**.VTG, PVTEST**M**.LAY	Multiskan EX/RC/MS

Copy the files corresponding to the test instrument and all the eight STDxxx.TXT files to the hard disk of your PC. The files must be copied on the same directory, where Ascent Software® is installed.

Note *Never use the original files of the diskette directly to run the test. Save the diskette files always as backup.*

Note *The filename STDxxx.TXT must be in this format. DO NOT change the name.*

Note *In the Windows® settings, the decimal separator must be a point (for example, 1.5) to ensure that the calculations are made correctly.*

Note *After recalibration, always copy the new reference absorbance files to the directory indicated above. The PVT Session files do not need to be copied unless a new version of the Session is sent.*

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3. How to perform the PVT test

These instructions assist the user to automatically verify the photometric performance using the PVT Session and the Ascent Software®. It is assumed that the user is familiar with Windows® and Ascent Software®.

3.1. Check list before start

- Warm up the instrument, see the User Manual of the instrument:

  - Multiskan Ascent®: 0 min
  - iEMS Reader MF: 0 min
  - Multiskan EX: 5 min
  - Multiskan RC: 5 min
  - Multiskan MS, Multiskan MCC 340 MK II, Multiskan Multisoft, Multiskan Bichromatic : 10-15 min
  - iEMS Analyzer : 0 min

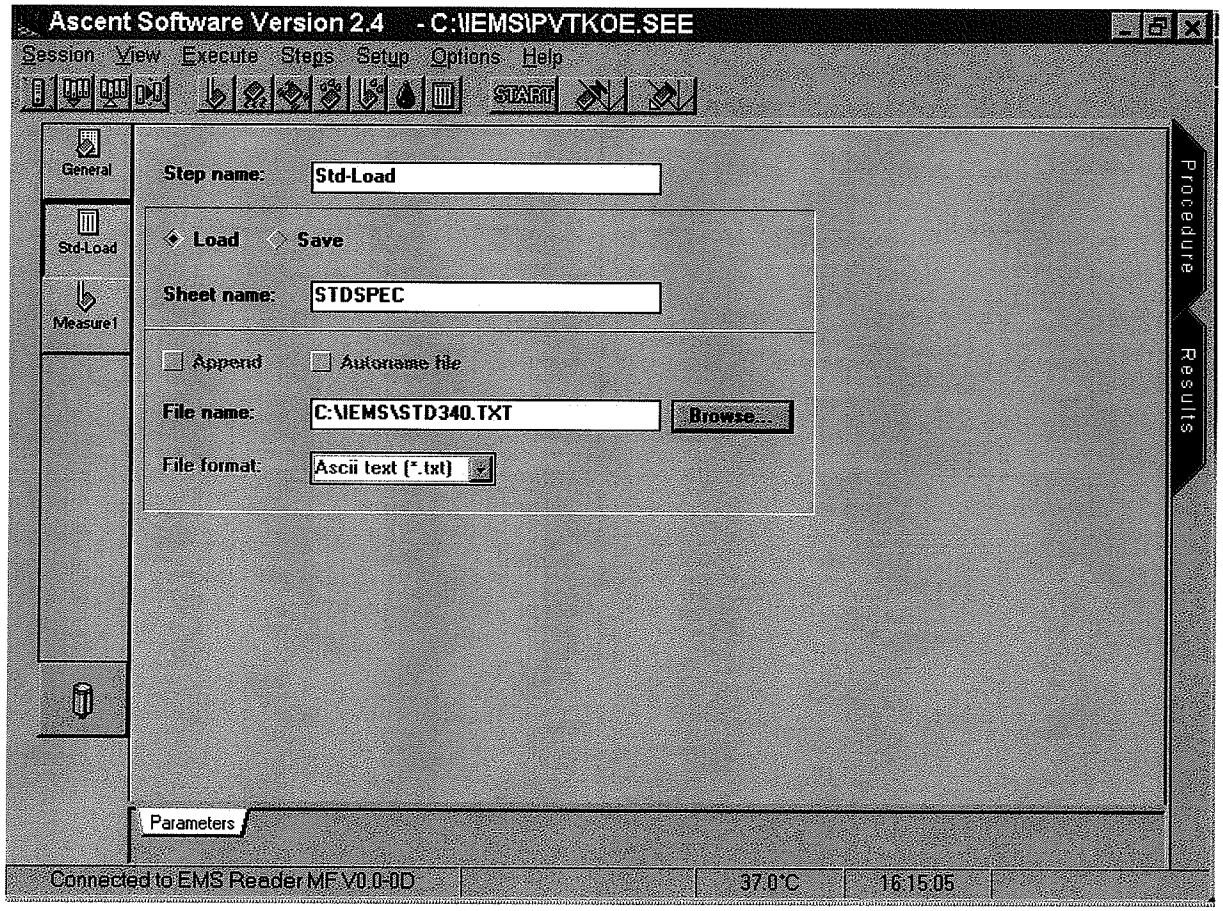
- Check that the serial number of the PVT Diskette is the same as that on the Verification Plate.

- Check visually that the NDF glasses are clean. If not, see the cleaning instructions on page 17.

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3.2. Running the PVT test

When the Ascent Software® is started, select *Open* from the *Session* menu and load the Session named PVTEST.sed (Multiskan Ascent®), PVTEST**E**.see (iEMS Reader MF) or PVTEST**M**.see (Multiskan EX/RC/MS) into your PC. On the left hand side of the **Procedure desktop** (shown below) you can see the steps of the PVT Session: General step; Load step (named '**Std-Load**'), and one or two measurement steps (named '**Measure1**' and '**Measure2**').



- Select one of the STDxxx.TXT files into the Std-Load step using the *Browse* key: see the dialog in the Procedure desktop above. This enables the Session to load the absorbance values into the STDSPEC spreadsheet in the Results desktop.

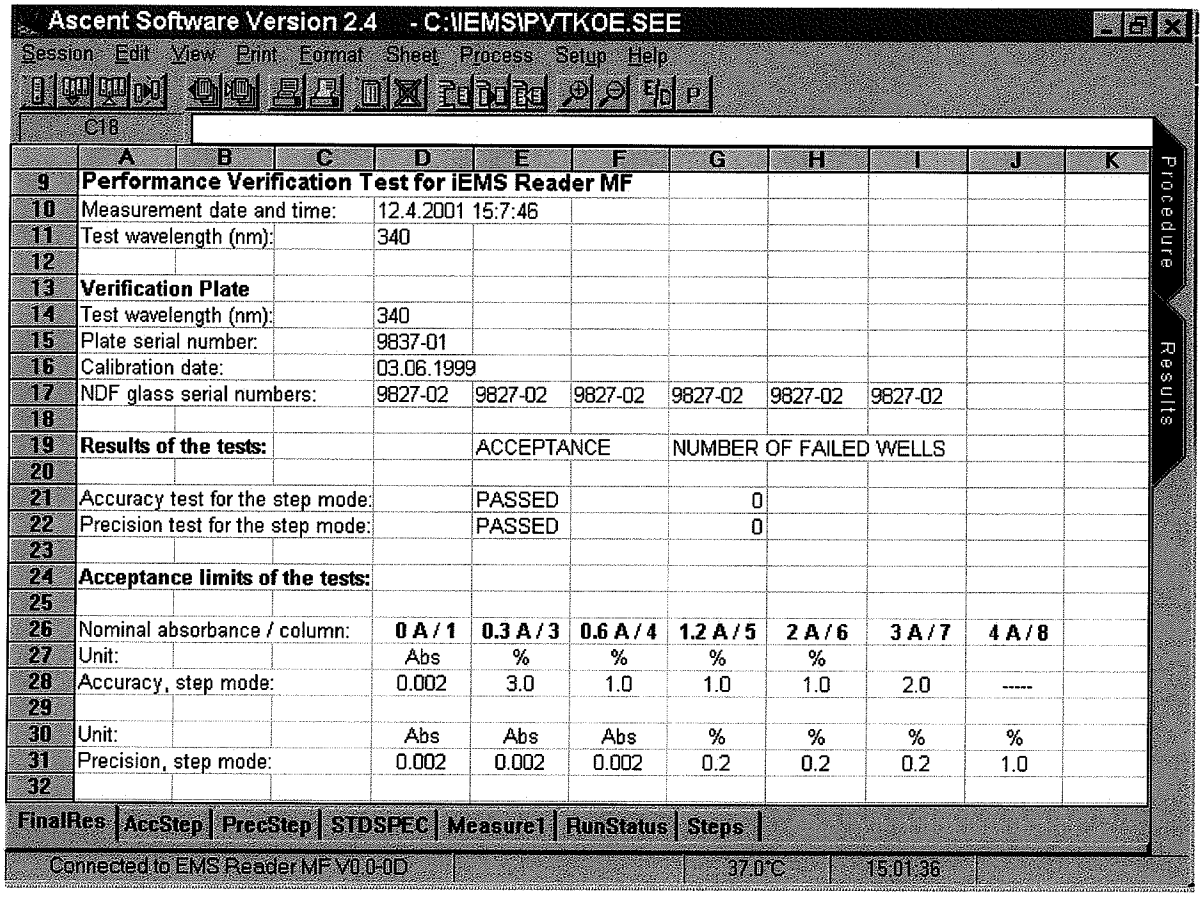
- Check that the wavelength in the **Measure** step(s) correspond(s) to the filename in the Std-Load step.

- Place the Verification Plate onto the plate carrier of the instrument and start the run. The PVT Session performs the verification test for one wavelength at a time. The instrument measures the Verification Plate in a 20-point measurement either once (**Measure1**) or twice (**Measure1** and **Measure2**).

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3.3. Reading the test results

When the measurement is ready, the **Results desktop** will appear on the screen with the following spreadsheets accessible on the bottom, for example, FinalRes, AccStep, PrecStep, STDSPEC, Measure1, RunStatus and Steps (see the table on Results desktop spreadsheets below on page 11).



	A	B	C	D	E	F	G	H	I	J	K
9	**Performance Verification Test for iEMS Reader MF**										
10	Measurement date and time:			12.4.2001 15:7:46							
11	Test wavelength (nm):			340							
12											
13	**Verification Plate**										
14	Test wavelength (nm):			340							
15	Plate serial number:			9837-01							
16	Calibration date:			03.06.1999							
17	NDF glass serial numbers:			9827-02	9827-02	9827-02	9827-02	9827-02	9827-02		
18											
19	**Results of the tests:**				ACCEPTANCE		NUMBER OF FAILED WELLS				
20											
21	Accuracy test for the step mode:				PASSED		0				
22	Precision test for the step mode:				PASSED		0				
23											
24	**Acceptance limits of the tests:**										
25											
26	Nominal absorbance / column:			**0 A / 1**	**0.3 A / 3**	**0.6 A / 4**	**1.2 A / 5**	**2 A / 6**	**3 A / 7**	**4 A / 8**	
27	Unit:			Abs	%	%	%	%			
28	Accuracy, step mode:			0.002	3.0	1.0	1.0	1.0	2.0	-----	
29											
30	Unit:			Abs	Abs	Abs	%	%	%	%	
31	Precision, step mode:			0.002	0.002	0.002	0.2	0.2	0.2	1.0	
32											

The **Measure1** sheet opens as default, but you can easily access the **FinalRes** sheet (see the **FINALRES** description below) by pressing the name of the sheet on the bottom of the screen.

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FinalRes | AccStep | AccCont | PrecStep | PrecCont | Measure1 | Measure2 | RunStatus | Steps | STDSPEC

Spreadsheet in Results desktop	*Description*
FinalRes	**Final test report per tested wavelength** - Laboratory and user information - Identification of the instrument, Verification Plate and NDF glasses - Date of calibration - Final test results with PASS/FAIL information - Acceptance limits for accuracy and precision tests
STDSPEC	**Source of the reference absorbance data per tested wavelength at six absorbance levels** – Loaded into the Std-Load step before measurement – Eight values per each absorbance level to correspond to the eight well positions in a microplate row – Identification of the Verification Plate and NDF glasses
Measure1	**Raw measurement data** - Measurement with Stepping mode (Multiskan Ascent® and iEMS Reader MF) - Measurement with Continuous mode (Multiskan EX/RC/MS) - Opens as default when the measurement is ready
Measure2	**Raw measurement data** – Measurement with Continuous mode (Multiskan Ascent®)
AccStep	**Calculations for the accuracy tests when Stepping mode was used** - Percentage difference from the reference value (STDSPEC sheet) calculated for eight well positions at six absorbance values - Acceptance for each well position (0 = PASS, 1 = FAIL), see equation 1 on page 14
AccCont	**Calculations for the accuracy tests when Continuous mode was used** - Percentage difference from the reference value (STDSPEC sheet) calculated for eight well positions at six absorbance values - Acceptance for each well position (0 = PASS, 1 = FAIL), see equation 1 on page 14

Continued

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FinalRes | AccStep | AccCont | PrecStep | PrecCont | Measure1 | Measure2 | RunStatus | Steps | STDSPEC

Spreadsheet in Results desktop	*Description* *Cont.*
PrecStep	**Calculations for the precision tests when Stepping mode was used** - All 20 measurements from one well position organized into one column - Standard deviation (SD), average (Mean) and CV% are calculated for each well position - Acceptance for each well position (0 = PASS, 1 = FAIL), see equation 1 on page 15
PrecCont	**Calculations for the precision tests when Continuous mode was used** - All 20 measurements from one well position organized into one column - Standard deviation (SD), average (Mean) and CV% are calculated for each well position - Acceptance for each well position (0 = PASS, 1 = FAIL), see equation 1 on page 15
RunStatus and Steps	Measurement information

Note *Never edit those cells in the spreadsheets that contain formulas and constants for calculations, because this can invalidate the test results.*

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3.4. Saving the test results

If you wish to save one sheet (for example, **FinalRes**) only, select *Save As* from the *Sheet* menu in the Results desktop and copy the sheet with a name of its own onto a separate file. If you wish to save the whole session, select *Save As* from the *Session* menu in the Results desktop and copy the session with a name of its own onto a separate file.

3.5. Printing the test results

Activate the area to be printed and select *Print Area/OK* from the *Print* menu in the Results desktop.

4. Manual Verification test

- Before starting read the check list (3.1) on page 8.
- Read the Verification Plate 20 times at each eight wavelength. To make it more convenient, you may use the kinetic mode with 20-point measurements. Use the first reading to calculate the photometric accuracy and all 20 readings to calculate the photometric precision.
- Photometric accuracy is verified manually by calculating the percentage difference of each reading from the reference value (see equation 1 for testing accuracy on p. 14) at eight well positions and at six absorbance values. The reference absorbance values are found in the Certificate of Calibration or in the PVT Diskette as STDxxx.TXT files (xxx corresponds to the wavelength). To make data handling more convenient, the STDxxx.TXT files can easily be inserted into the spreadsheet programs. For this reason, the files have been stored in user-friendly, tab-separated ASCII text format.
- Photometric precision can be verified manually by calculating the CV% for each well position as described on pages 15 (equation 1) and 18.

Note *You may change the number of readings of the Verification Plate from the number stated here (20 times), depending on the requirements of your own QC system.*

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5. Interpretation of the results

5.1. Accuracy

Accuracy is determined for eight wavelengths at six absorbance levels, each of which covers eight well positions in a row. A single reading (the first reading of the 20-point measurement) of the Verification Plate is compared to the accuracy criteria as follows:

(1) $$\frac{\text{Test value} - \text{Reference absorbance value}}{\text{Reference absorbance value}} \times 100 < \text{Accuracy criteria}$$

(2) ***Note*** All readings must meet the accuracy criteria to pass the accuracy test.

Accuracy criteria

The accuracy criteria at absorbance level 0.6 – 2.0 Abs/4.0 Abs

The accuracy criteria are equal to the accuracy specifications of the instrument (see the User Manual of the instrument) excluding the lowest absorbance level 0.3.

The accuracy criteria at absorbance level 0.3 Abs

Measurement uncertainty is a result of the number of related components used in the measurement: the calibration equipment; the NDF glasses of the Verification Plate, and the performance (+ the filters) of the test instrument. In theory the correct accuracy criteria corresponds to the accumulated uncertainty caused by all of the above-mentioned factors. In other words, setting the criteria to the level of uncertainty attributable to the instrument places greater demands on the instrument than what has been specified.

At absorbance level 0.3, even the smallest dust particles and scratches on the NDF glass surfaces are easily detected. That is why the accumulated uncertainty (3%) has been chosen as the acceptance criteria at 0.3 Abs.

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5.2. Precision

Precision, i.e., coefficient of variation (CV%), is determined for any well position after reading the Verification Plate 20 times. The CV% is calculated as follows:

(1) $$CV\% = \frac{SD}{MEAN} \times 100$$, where

SD = the standard deviation of all measurements
MEAN = the average of all measurements.

(2) ***Note*** All well positions must meet the precision criteria to pass the precision test
CV% < Precision criteria

Precision criteria

The precision criteria at absorbance level 0.3 – 2.0 Abs/4.0 Abs

The precision criteria is equal to the precision specifications of the instrument, see the User Manual of the instrument.

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6. Troubleshooting

If the results for both the accuracy and the precision tests are "PASSED", the instrument has been verified to give correct measurement data. In case of any problems, the reason may be a faulty or unclean instrument or Verification Plate. Generally, if one or more of the precision tests have FAILED, the reason will probably lie in the instrument. If one or more of the accuracy tests have FAILED, the reason may lie in the instrument or in the plate. There are some factors below, which should then be checked:

- If all the results are considerably out of range:
  - Check that the Verification Plate has been placed correctly onto the plate carrier.
- If all the results are only slightly out of range:
  - Check that the wavelength in the PVT Session (Std-Load step and Measure step(s)) is correct.
  - Check that the filters in the instrument are in the proper order with regard to the filter wavelengths.
- If all the results of some glasses are out of range:
  - Check that the NDF glass positioning and the order are correct by dismantling the Verification Plate as shown in *Fig. 2* on page 17.
- If the results are only randomly out of range:
  - Clean the optic lenses, filters (see the User Manual of the instrument) and the NDF glasses of the Verification Plate (see section 8. Cleaning on page 17).
  - Check that the lamp is working.
- If the fault does not disappear, contact your service personnel or the service company you use.

7. Storage

Always store the Verification Plate in its own case when not in use to keep the glass surface free of dust. In general, avoid sites of storage with excessive dust, dirt, moisture or large temperature fluctuations.

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8. Cleaning

If you can visually see dust particles on the surface of NDF glasses, remove the dust with pure compressed air. If you see any spillages, clean the glasses with a tissue moistened with ethanol (96%), rinse the glasses with distilled water and dry them well.

To make cleaning thorough, dismantle the Verification Plate as shown in *Fig. 2*. When assembling, be careful to place the NDF glasses into the Verification Plate with the absorbance value marking as indicated below (*Fig. 2*).

Note *Handle the NDF glasses with care. DO NOT ever use any cleaning materials that may scratch the glasses.*

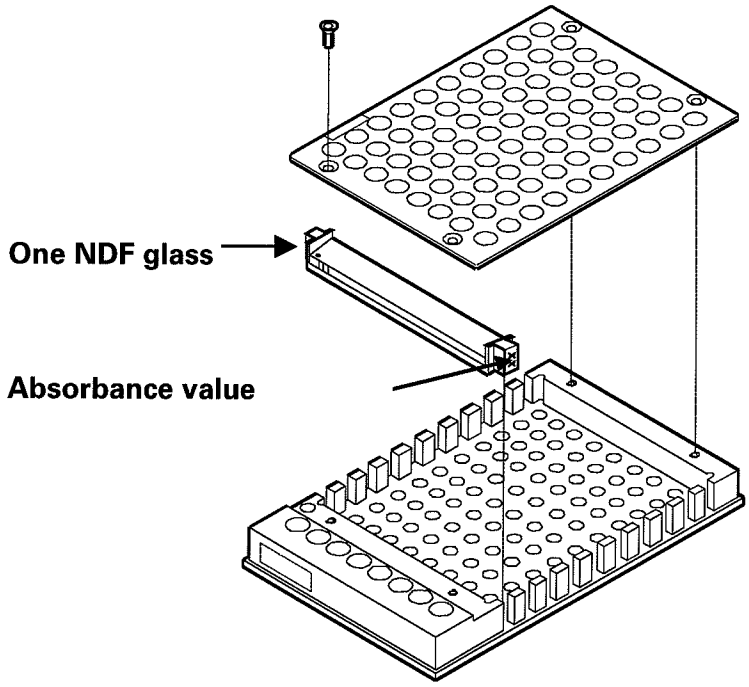


Fig. 2 Assembly drawing of the Verification Plate

9. Repair

Thermo Electron does not deliver or accept any separate parts for recalibration or repair. Faulty NDF glasses can only be replaced at Thermo Electron.

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10. Glossary and abbreviations

absorbance = Negative logarithm of one minus absorptance as measured on a uniform sample. *Abbr.* Abs or A

accuracy =
1) Accuracy is a complex concept which reflects the ability of the assay to measure the true value of the analyte. Accuracy means correctness; freedom from error. The accuracy of results can be measured by comparing them to results accepted as correct, or by comparing them with those from another laboratory (this is 'relative accuracy').
2) Deviation of the mean value from the nominal value.

coefficient of variation = The measure of variability. CV is expressed as a percent and is calculated as:

$$CV(\%) = \frac{SD}{MEAN} \times 100, \text{ where}$$

SD = the standard deviation of all measurements
MEAN = the average of all measurements.

NDF = Neutral Density Filter

NPL = National Physical Laboratory

objective evidence = Information which can be proved true, based on facts obtained through observation, measurement, test or other means.

precision = The measure of the closeness of the results obtained when analyzing the same sample more than once; the measure of agreement between replicate measurements.
In statistical quality control, the repeatability of a measurement, as measured by the variance of repeated measurements.

PVT = Performance Verification Test

standard deviation (SD) = A quantitative measure of how individual values are distributed about the mean. Standard deviation is calculated using the following formula:

$$\text{Standard Deviation (SD)} = \sqrt{\frac{\text{Sum of (Individual Value} - \text{Mean)}^2}{\text{Number of Values} - 1}}$$

validate =
1) To prove to be valid; confirm the validity of.
2) To make binding under the law; give legal force to; declare legally valid.
3) To check that an input or data is correct according to a set of rules.

validation = Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled. In other words, a check performed to validate data. The process of evaluating the performance of a specific measuring procedure and checking that the performance meets certain preset criteria. Validation establishes and provides documented evidence that the measuring procedure is fit for a particular purpose.

Continued

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verification = Confirmation by examination and provision of objective evidence that specified requirements have been fulfilled.

***lay** = File that contains all session layouts.

***sed** = Multiskan Ascent® session file

***see** = iEMS Ascent session file

***txt** = Text file in ASCII format.

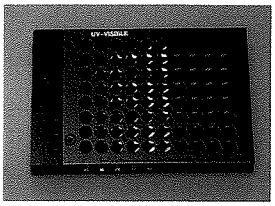
***vtb** = File that contains all session sheets.

***vtg** = File that contains all session graphs.

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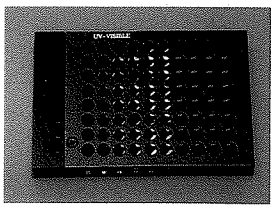
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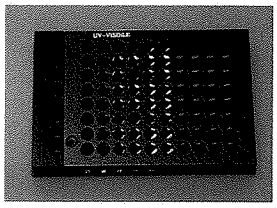
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