



TITLE: Vaccine Vial Monitor

Product verification protocol: E06/IN05.VP.2
Applies to specification ref(s): E06/IN05.2
Issue date: 9 May 2011
Date of last revision: 20 November 2006

Contents:

1. Scope: 1

2. Normative references: 2

3. Terms and definitions:..... 2

4. Applicability: 3

5. Type-testing procedure: 3

5.2 Test procedure:..... 3

5.2.1 VVM storage and handling during testing: 3

5.2.2 Test conditions:..... 3

5.2.3 Colour measurements: 4

5.2.4 Reaction rates:..... 4

5.2.5 Test 1: Format and dimensions of VVMs: 4

5.2.6 Test 2: Characterizing the colour change over time: 4

5.2.7 Test 3: Recording the start point: 5

5.2.8 Test 4: VVM reaction rate; +37°C, no light: (applies to all VVM categories): 6

5.2.9 Test 5: VVM reaction rate; +37°C, 75% RH, no light: (applies to all VVM categories) 6

5.2.10 Test 6: VVM reaction rate; +37°C, 33% RH, no light: (applies to all VVM categories) 7

5.2.11 Test 7: VVM reaction rate; +25°C, no light: (applies to VVM30, VVM14 and VVM7 categories only)..... 7

5.2.12 Test 8: VVM reaction rate; +5°C, no light: (applies to VVM2 category only)..... 7

5.2.13 Test 9: Reversion test: (applies to all VVM categories) 8

5.2.14 Test 10: Soak test: (applies to all VVM categories) 8

5.2.15 Test 11: Observer perception test: (applies to all VVM categories)..... 8

5.3 Test criteria for qualification: 8

6. Quality control checklist: 9

6.1 Quality control standards: 9

6.2 Quality control checklist: 9

7. Pre-qualification evaluation:..... 9

8. Modified products:..... 9

9. Annexes:..... 9

Revision history:..... 10

1. Scope:

This document describes the procedure for verifying the performance of vaccine vial monitors.

2. Normative references:

ISO/IEC 17025: 2005 *General requirements for the competence of testing and calibration laboratories*.

WHO/PQS /E06/IN05.2: *WHO Performance Specification for Vaccine Vial Monitors*.

3. Terms and definitions:

Active surface: A time-temperature sensitive colour patch whose **reaction rate** closely matches the stability profile of the vaccine to which the **VVM** is attached¹.

End point: The point at which time-temperature exposure has altered the colour of the **active surface** so that it exactly matches the **reference surface**. At this point, and thereafter, the vaccine is no longer suitable for use and should no longer be used.

In writing: means communication by letter, fax or email.

Legal Manufacturer: The natural or legal person with responsibility for the design, manufacture, packaging and labelling of a product or device before it is placed on the market under his own name, regardless of whether these operations are carried out by that person himself or on his behalf by a third party.

OD: Optical Density.

Reference surface: A colour patch against which the colour of the **active surface** can be directly compared.

Reaction rate: The rate at which the **active surface** responds to time-temperature exposure.

Reseller: A commercial entity, licensed to act on behalf of a **Legal Manufacturer**, and which carries product liability and warranty responsibilities no less onerous than those carried by the Legal Manufacturer.

Spectrodensitometer: The specification for the Start R-I, Indicator OD values, Reference Ring, and OD limits found in **E06/IN05.2** are based on measurements with an X-Rite Model 500 series spectrodensitometer.

Measurements taken with other instrumentation will require a conversion factor. Due to the small size of the VVM's reference ring and indicator area, it is necessary to modify the target and aperture centring of the spectrodensitometer (as sold by the instrument supplier). The VVM manufacturer will be responsible for providing the service to install the target and centre the aperture. Conversion of spectral data to optical density is defined within ISO 5-3:1995 Photography-Density measurements-Part 3: Spectral Conditions.

Start point: The colour of the **active surface** of the VVM at the time when the VVM is received by the vaccine manufacturer².

¹ It is the vaccine manufacturer's responsibility to match the stability profile of their vaccine to the time-temperature profile of one of the four VVM types described in clause 4.2.8 of this specification.

² It is the vaccine manufacturer's responsibility to store the VVMs correctly to prevent any change in the start OD during the period elapsing between the time of receipt of the VVM to the time of application to the filled vaccine vial.

VVM: Vaccine Vial Monitor comprising, as a minimum, an **active surface**, a **reference surface** and the substrate to which these are applied by the VVM manufacturer.

4. **Applicability:**

Type testing must be carried out by an independent **ISO/IEC 17025** accredited testing laboratory, pre-qualified by WHO. On-site inspection of the **legal manufacturer's** production facilities will be carried out by WHO or by a consultant appointed by WHO for this purpose.

5. **Type-testing procedure:**

5.1 ***Number of samples:*** The **Legal Manufacturer** or **Reseller** must supply the testing laboratory with a full duplicate set of the Product Dossier already supplied to WHO in accordance with the requirements of specification clause 7. The following test samples are required for each VVM reaction rate category to be tested:

- 500 VVMs.
- Six test patches of the **active surface**. The 'test patches' of **active surface** must be at least 7 mm diameter, printed on the same backing paper as the VVM, but without the printed **reference surface**.

All samples must be in an active state. They must be packed in an insulated container with dry ice or frozen gel packs and there must be residual dry ice or partially frozen gel packs in the container when it arrives at the laboratory. The VVMs and test patches must be clearly labelled with individual identification numbers and the relevant reaction rate category (2, 7, 14 or 30).

5.2 ***Test procedure:***

5.2.1 ***VVM storage and handling during testing:***

- **Storage:** The VVMs and test patches must be stored below -24°C in a freezer whose temperature is recorded continuously. Testing must commence within two weeks of the arrival of the samples at the laboratory.
- **Handling:** When the samples are handled in preparation for the tests they must be removed from freezing, individually, and for the briefest period possible, before being returned again to storage below -24°C.

5.2.2 ***Test conditions:***

- **Monitoring the test environment:** The temperature of the water bath and the temperature and relative humidity of the test chamber must be monitored throughout the test and a summary of the data included in the final report.
- **Temperature stability:** The temperature stability and uniformity of the water bath and of the test chamber must be controlled within a tolerance of $\pm 0.2^\circ\text{C}$ and the relative humidity must be controlled within a tolerance of $\pm 5\%$ RH.
- **Humidity:** Humidity in the test chamber must be controlled accurately with salt solutions, e.g., 33% RH - 370 g of Magnesium Chloride hexahydrate per 100 g of de-ionized water and 75% RH - 45 g of Sodium Chloride per 100 g of de-ionized water.

- 5.2.3 *Colour measurements:* Colour Measurements must be made with a Spectraflash SF600 Spectrophotometer or equivalent and with a X-Rite 500 series [spectrodensitometer](#), or as agreed with the manufacturers of the VVMs.
- 5.2.4 *Reaction rates:* [Reaction rates](#) are specific to four different categories of VVM, relating to four groups of vaccines according to their heat stability at two specific temperature points (See Table 1).

Table 1: VVM reaction rates by category of heat stability and temperature and time periods for testing.

Category (Vaccines)	No. of days to end point at +37°C	No. of days to end point at +25°C	Time to end point at +5°C
VVM 30: High Stability	30	193	> 4 years
VVM 14: Medium Stability	14	90	> 3 years
VVM 7: Moderate Stability	7	45	> 2 years
VVM 2: Least Stable	2	N/A*	225 days

*VVM (Arrhenius) reaction rates determined at two temperature points

Unless otherwise specified, the two temperatures and time periods, highlighted in Table 1 for each VVM category, will be the agreed test period for testing each category. Additionally, each VVM category will be tested at a time greater than the [end point](#) time to verify that all VVM samples will reach the [end point](#).

- 5.2.5 *Test 1: Format and dimensions of VVMs:* Check the dimensions of the VVMs against the limits set out in specification **E06/IN05.2**, clause 4.2.1.

The following procedure must be adopted:

- **Measurement:** For each VVM category to be tested, a random sample of 20 VVMs of each category must be used to check the ratio of the square area to the reference circle area.
- **Sample handling:** When the samples are handled for measurement, they must be individually removed from freezing, for the briefest possible period, before being returned again to storage below -24°C.

- 5.2.6 *Test 2: Characterizing the colour change over time:* (Note required only one time for the product family) Specification **E06/IN05.2**, clause 4.2.2, requires a shade change without a hue change. It is therefore essential to study the colour change using both a spectrophotometer/colorimeter and [spectrodensitometer](#) to ensure that the extent of hue change is small enough not significantly to affect the validity of the colour densitometer readings, which are used for the remainder of the tests.

The following procedure must be adopted:

- **Incubation:** Incubate three test patches for each VVM category to be tested. The incubation procedure must be carried out at +37°C ±0.2°C inside sealed pouches (5-mil heat sealable foil polyethylene polyester MIL-B-131, Class 1 Type 1) or equivalent, pouches measuring 150mm x 200mm) in a water bath.

- **Readings:** Readings must be taken at time zero, and at the same time each day, until the [end point](#) is reached. Remove the sealed pouch(es) from the water bath and extract the test patch samples immediately before taking the readings.

Each reading must consist of one measurement taken with the spectrophotometer, and one measurement taken with the colour reflection densitometer, positioning the instrument heads at the centre of the test patch.

All readings must be taken at room temperature, in the shortest possible time. Afterwards, the test patches must immediately be placed back into the sealed pouch(es) and returned to the water bath.

The readings must be taken for each of the three samples in each VVM category.

- **Tabulation:** The [spectrodensitometer](#) readings must be tabulated alongside the spectrophotometer readings. The difference in L value between the start point and that measured on each day of the test must be plotted against the corresponding change in optical density. If these data do not correlate, then the results must be discussed with WHO before proceeding with the remaining tests.

5.2.7 Test 3: Recording the [start point](#):

- **Sample size:** 500 for each VVM category.
- **Initial procedure:**
 - Using a [spectrodensitometer](#), read and record the same portion of the [active surface](#) on all 20 VVM samples for each category.
 - Take five of the samples in each category, read and record three different portions of the [reference surface](#), and calculate the average reading for each sample.
 - Tabulate the readings.
- **Rejection/acceptance criteria:** If the following conditions are met the next stage of the test may proceed. If one or more of the conditions are *not* met the manufacturer must be asked to submit a new batch of sample VVMs for testing.
 - The active surface readings for all each of the 20 samples must be within ± 0.03 OD of the mean for the whole group.
 - When five samples are selected at random, the difference between the readings for the [active surface](#) and for the [reference surface](#) of each category must conform to the specification for the [start point](#) set out in **E06/IN05.2**, clause 4.2.4.
 - The difference between the readings of the active surface and the reference surface (Start R-I) of each category should conform to the specification for the start point set out in **E06/IN05.2**, clause 4.2.4. when five samples are selected at random.
 - The Indicator OD measurements at the start point of each category should conform to the specification set out in **E06/IN05.2**, clause 4.2.4. when five samples are selected at random.
 - The three readings taken from the reference circle on five samples should conform to the specification for homogeneity set out in **E06/IN05.2**, clause 4.2.5.
 - The colour density of one portion of the reference ring compared to the colour portion of the reference ring when five samples are selected at

random should conform to the variation of the reference surface specification set out in **E06/IN05.2**, clause 4.2.6.

- The Reference Ring OD measurements at the start point of each category should conform to the specification set out in **E06/IN05.2**, clause 4.2.7 when five samples are selected at random.
- The specification for the Start R-I and the Indicator OD values, the Reference Ring Specification and OD limits found in **E06/IN05.2** are based on measurements with an X-Rite Model 500 [spectrodensitometer](#) calibrated by white calibration patch specific to instrument serial number. Measurements taken with other instrumentation will require a conversion factor.
- The three readings taken from the reference circle on five samples must conform to the specification for homogeneity set out in **E06/IN05.2**, clause 4.2.5.
- If all the above conditions *are* met, the samples should be divided into four sets (I, II, III, and IV) of 60 samples each for the reaction rate tests described in clauses 5.2.8 through 5.2.12. The remainder of the samples will be used as described in clauses 5.2.13 through 5.2.15. All samples must be stored below -20°C until testing begins.

5.2.8 *Test 4: VVM reaction rate; $+37^{\circ}\text{C}$, no light: (applies to all VVM categories):*

- **Step 1:** Expose sample set 'I' (60 samples) to $+37^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ inside sealed foil polyethylene MIL-B-131, Class 1 Type 1) or equivalent, pouches in a water bath, without light. Remove the relevant sealed pouch(es) from the water bath and extract the test patch samples immediately before taking the readings referred to below.
- **Step 2:** After 75% of the agreed test period in the water bath, remove the first portion (20 VVMs) from the bath. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results. Afterwards, the test patches must immediately be placed back into the sealed pouch(es) and returned to the water bath.
- **Step 3:** After completion of the agreed test period, remove the second portion (20 VVMs) from the bath. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results. Afterwards, the test patches must immediately be placed back into the sealed pouch(es) and returned to the water bath.
- **Step 4:** After 125% of the agreed test period in the water bath, remove the third portion (20 VVMs) from the bath. Using a [spectrodensitometer](#) read the [active surface](#) on all 20 samples and record the results. Afterwards, the test patches must immediately be placed back into the sealed pouch(es) and returned to the water bath.
- **Step 5, for checking purposes:** Following steps 1 to 4, continue to store the set 'I' samples at $+37^{\circ}\text{C}$ for an additional 30 days. Using a [spectrodensitometer](#), re-read the [active surface](#) on all samples and compare the results with those taken at the end of Step 4. Record the results of the comparison.

5.2.9 *Test 5: VVM reaction rate; $+37^{\circ}\text{C}$, 75% RH, no light: (applies to all VVM categories)*

- **Step 1:** Expose sample set 'II' (60 samples) to $+37^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ in a temperature-controlled cabinet, without light and at a relative humidity of $75\% \pm 5\%$.

- **Step 2:** After 75% of the agreed test period in the cabinet, remove the first portion (20 VVMs) from the cabinet. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.
- **Step 3:** After completion of the agreed test period, remove the second portion (20 VVMs) from the cabinet. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.
- **Step 4:** After 125% of the agreed test period in the cabinet, remove the third portion (20 VVMs) from the cabinet. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.

5.2.10 *Test 6: VVM reaction rate; +37°C, 33% RH, no light: (applies to all VVM categories)*

- **Step 1:** Expose sample set 'III' (60 samples) to +37°C ±0.2°C in a temperature-controlled cabinet, without light and at a relative humidity of 33% ±5%.
- **Step 2:** After 75% of the agreed test period in the cabinet, remove the first portion (20 VVMs) from the cabinet. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.
- **Step 3:** After completion of the agreed test period, remove the second portion (20 VVMs) from the cabinet. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.
- **Step 4:** After 125% of the agreed test period in the cabinet, remove the third portion (20 VVMs) from the cabinet. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results. Move these samples to 5°C storage (see Reversion Test, clause 5.2.13).

5.2.11 *Test 7: VVM reaction rate; +25°C, no light: (applies to VVM30, VVM14 and VVM7 categories only)*

- **Step 1:** Expose sample set 'IV' (60 samples) to +25°C ±0.2°C inside sealed foil polyethylene MIL-B-131, Class 1 Type 1) or equivalent, pouches in a water bath, without light. Remove the relevant sealed pouch(es) from the water bath and extract the test patch samples immediately before taking the readings referred to below.
- **Step 2:** After 60% of the agreed test period in the water bath, remove the first portion (20 VVMs) from the bath. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.
- **Step 3:** After completion of the agreed test period, remove the second portion (20 VVMs) from the bath. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.
- **Step 4:** After 140% of the agreed test period in the water bath, remove the third portion (20 VVMs) from the bath. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.

5.2.12 *Test 8: VVM reaction rate; +5°C, no light: (applies to VVM2 category only)*

- **Step 1:** Expose sample set 'IV' (60 samples) to +5°C ±0.2°C inside sealed foil polyethylene MIL-B-131, Class 1 Type 1) or equivalent pouches in a water bath, without light. Remove the relevant sealed pouch(es) from the water bath and extract the test patch samples immediately before taking the readings referred to below.
- **Step 2:** After 60% of the agreed test period in the water bath, remove the first portion (20 VVMs) from the bath. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.

- **Step 3:** After completion of the agreed test period, remove the second portion (20 VVMs) from the bath. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.
- **Step 4:** After 140% of the agreed test period in the water bath, remove the third portion (20 VVMs) from the bath. Using a [spectrodensitometer](#), read the [active surface](#) on all 20 samples and record the results.

5.2.13 *Test 9: Reversion test: (applies to all VVM categories)*

- **Step 1:** Following on from 5.2.2 10 Step 4, store the third portion of 20 samples from set 'III' at $+5 \pm 0.2^\circ\text{C}$ for 30 days.
- **Step 2:** Re-read the active surface of the samples with a [spectrodensitometer](#) and compare the readings with those taken at the end of the first test.

5.2.14 *Test 10: Soak test: (applies to all VVM categories)*

- **Step 1:** Adhere two groups of 10 VVM labels to water impermeable substrates (e.g., white plastic picnic plates).
- **Step 2:** Submerge the first group in a water bath at $+5 \pm 0.2^\circ\text{C}$ for 8 hours. Seal the second (dry) group of labels in foil polyethylene MIL-B-131, Class 1 Type 1) or equivalent waterproof pouches and subject to the same temperature treatment.
- **Step 3:** At the end of the 8-hour period, remove the labels from the water bath and carefully dry the soaked labels with absorbent towels.
- **Step 4:** Place both groups in a desiccant chamber at $+5 \pm 0.2^\circ\text{C}$ for 16 hours.
- **Step 5:** Remove both groups from the desiccant chamber and place in two foil polyethylene MIL-B-131, Class 1 Type 1) or equivalent waterproof pouches in a $+37 \pm 0.2^\circ\text{C}$ water bath. Once a day, remove the labels from the water bath and measure the active portion of each VVM until each reaches the [end point](#).
- **Step 6 – evaluation:** The results of the soaked versus dry [OD](#) measurements should be compared for conformity to **E06/IN05.2**, clause 4.2.4 (less than a 0.04 OD unit difference).

5.2.15 *Test 11: Observer perception test: (applies to all VVM categories)*

- **Step 1:** Attach 15 VVM samples to empty 2 ml vials. Five of the VVM samples should be at the [start point](#), five should be conditioned to 50% of the colour change to end point and five should be at the [end point](#).
- **Step 2:** Place the samples in a box in a random order and store in a freezer below -24°C to prevent further colour change.
- **Step 3 – evaluation:** Five untrained observers, working independently under tungsten or fluorescent light at 100 lux on the working plane, are to sort the vaccine vials into three groups (unchanged, 50% changed, and end point). Record the level of the light used.
- **Acceptance criterion:** All five observers are able to sort the three groups of vials with 100% accuracy.

5.3 Test criteria for qualification:

A final report must be issued after all testing is complete. The report of the tests must contain the following data and analyses:

- **Generally:** Water bath and test chamber temperature and humidity records.
- **Test 1:** Dimensional tolerances of the VVM.

- **Test 2:** Characterization of the colour change - table of readings and plot of L vs. OD.
- **Test 3:** Distribution of active surface starting point readings - maximum, minimum and mean.
- **Test 3:** Indicator readings at the start point – table of readings.
- **Test 3:** Distribution of the difference between the active surface and the reference surface starting point readings - maximum, minimum and mean.
- **Test 3:** Homogeneity readings from the reference surface - table of readings.
- **Test 3:** Variability readings from the reference ring – table of readings.
- **Test 3:** Reference rings readings at the start point- table of readings.
- **Tests 4 to 8:** Distribution of reaction test readings at all temperatures and times - percent reaching the end point.
- **Test 9:** Reversion test.
- **Test 10:** Soak test.
- **Test 11:** Observer perception test.
- **Annexes:** Additional supporting documentation requested and received from the [Legal Manufacturer](#) or [Reseller](#) during the course of the type-testing.

6. Quality control checklist:

6.1 *Quality control standards:* All testing and reporting must be carried out in accordance with the requirements of [ISO 17025: 2005](#) or later edition.

6.2 *Quality control checklist:* An on-site inspection of the VVM manufacturing plant is required in accordance with clause 4.

7. Pre-qualification evaluation:

A product will qualify for inclusion on the register of PQS pre-qualified VVMs in accordance with WHO procedures provided the final report indicates full conformity with the requirements of specification **E06/IN05.2**.

8. Modified products:

The [legal manufacturer](#) or [reseller](#) must notify WHO **in writing** of any changes which affect the performance of the product in relation to any of the requirements set out in this verification protocol. WHO will carry out a desk evaluation of the reported change(s). If any change is deemed adversely to affect the performance of the product, WHO may request full or partial re-verification based on the test procedures described in this document.

9. Annexes: None

Revision history:			
Date	Change summary	Reason for change	Approved
14 Mar 2006	Test procedure redrafted with general amendments to the form of wording but not to the content. Normative references, definitions and additional clauses added.	To achieve conformity with PQS documentation standards	UK
30 Nov 2006	General revisions	Following consultation with industry	UK (30 November 2006 - PQS secretariat)
7 Apr 2011	2: ISO 17025 date corrected. 3: Spectrodensitometer definition added. 5.2.3: Spectrodensitometer model changed. 5.2.6: Densitometer changed to spectrodensitometer. 5.2.7: Ditto. 'Read and record the active surface' deleted. Spectrodensitometer model and calibration method changed. 5.2.8: Densitometer changed to spectrodensitometer. 5.2.9: Ditto. 5.2.10: Ditto. 5.2.11: Ditto. 5.2.12: Ditto. 5.2.13: Ditto.	Consultation with industry. Previous model no longer manufactured. Consistency. Consultation with industry Consistency.	UK 9(May 2011)