PQS Independent type-testing protocol

TITLE: Vaccine Vial Monitor

Product verification protocol:	E006/IN05.VP.3
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Contents

1. Scope

This document describes the procedure for verifying the performance for a *Vaccine Vial Monitor (VVM)*, which is designed to warn health workers when the cumulative time-temperature exposure of a vial of vaccine has exceeded a pre-set limit, beyond which the vaccine should not be used. Before the end point is reached, gradual shade changes in the VVM active surface can alert health workers that particular vials have been partially exposed. These vials can then be used in preference to those that have not been exposed. VVMs may also be supplied together on the same label with other indicators. Each indicator technology must comply with and be tested in accordance with their own appropriate specification and protocol.

2. Normative references

ISO/IEC 17025: 2005 General requirements for the competence of testing and calibration laboratories.
WHO/PQS /E006/IN05.2: WHO Performance Specification for Vaccine Vial Monitors.

3. Terms and definitions

Active surface: A time-temperature sensitive patch that has a reaction rate closely matching the stability profile of the vaccine to which the VVM is attached[1].
End point: The point at which time-temperature exposure has altered the shade of the active surface so that it matches the shade of the reference surface. At this point, and thereafter, the vaccine is no longer suitable for use.
In writing: 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 themselves or on their behalf by a third party.
Mean Kinetic Temperature (MKT): A single derived temperature that, if maintained over a defined period of time, affords the same thermal challenge to a drug substance or drug product as would be experienced over a range of both higher and lower temperatures for an equivalent defined period. The mean kinetic temperature is higher than the arithmetic mean temperature and takes into account the Arrhenius equation.
OD: Optical Density – reflected OD in the case of this specification. The logarithmic measure of light reflected from the surface of the VVM are measured by an appropriate instrument such as a spectrodensitometer or a densitometer. $OD = \log_{10} R$, where R is reported in decimal format
Reference surface: A patch against which the shade of the active surface can be directly compared.
Reaction rate: The rate at which the active surface responds to time-temperature exposure.

[1] In consultation with the WHO, the vaccine manufacturer should match the stability profile of their vaccine to the time-temperature profile of one of the VVM types described in PQS catalogue

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.
R – I: The reference surface OD value minus the active surface OD value.
Spectrodensitometer: Instrument to measure reflected optical density. Note that not all Spectrodensitometers have the ability to measure spectral data or display colorimetric information. Owing to the small size of the VVM's reference ring and indicator area, it is necessary to ensure the target and aperture centering of the spectrodensitometer is suitable for measuring the active surface and the reference surface. Conversion of spectral data to optical density is defined within ISO 5-3:2009 Photography-Density Measurements -Part 3: Spectral Conditions. All such instruments must be calibrated before each use with a certified reference tile.
Start point: The optical density of the active surface of the VVM at the time when the VVM is received by the vaccine manufacturer.[2]
Test patches: "Patches" of active surface *may be* supplied by the manufacturer.[3] They are at least 7 mm diameter, printed on the same backing paper as the VVM, but without the printed reference surface.
Vial: For the purposes of this verification protocol, a "vial" also refers to other primary containers containing vaccine onto which a VVM may be applied, for example droppers, ampules or pre-filled syringes.
VVM: Vaccine Vial Monitor comprising, as a minimum, an active surface, a reference surface and the substrate to which these are attached by the VVM manufacturer.

4. Applicability

Type testing must be carried out by an independent ISO/IEC 17025 accredited testing laboratory, prequalified 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.

[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.
[3] VVMs can be used instead of patches if the spectrodensitometer has an appropriate specification (i.e. small enough measurement area to fit entirely inside the active or reference surface).

5. Type-testing procedure

5.1 Sample control

The following test samples are required for each VVM reaction rate type to be tested:

- ✓ 500 VVMs, to enable the test laboratory to select random samples from the VVMs provided,
- ✓ Six test patches of the active surface *if* required by the testing laboratory. 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.

The VVMs and any test patches must be clearly labelled with individual identification numbers and the relevant reaction rate type: 2, 7, 11, 14, 30 or 250.

5.2 General test procedure

5.2.1 VVM transit, storage and handling

Transit: Samples supplied in an active state 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.

Storage: Before testing, active VVMs and test patches must be stored in temperatures at or below –24°C in a freezer whose temperature is recorded frequently. VVMs in cold storage should be packaged to avoid condensation and moisture contamination. Testing should commence within about two weeks of the arrival of the samples at the laboratory[4]. Active VVMs should be stored as specified by the manufacturer.

Handling: When active samples are handled in preparation for the tests they must be removed from the freezer in small subset quantities, for the briefest period possible before being returned again to storage at or below –24°C. For VVM samples which are supplied initially inactive, follow the manufacturer's instructions for storage and handling.

[4] This is to help track shipment or storage issues if early results show non-compliance.

5.2.2 Test conditions

Conditioning test samples: Activated test samples may be conditioned for tests either in an incubator or a water bath, providing temperatures and humidity can be kept within the tolerances specified below. Temperatures can be kept in close control in a water bath or a good incubator set up.

Temperature stability: The temperature of the water bath and/or the incubator must be maintained at the target Mean Kinetic Temperature (MKT) within a tolerance of ±0.2°C.[5] This temperature requirement should be verified by test environment validation or with an array of temperature sensors around the test sample area.

The temperature of the water bath should be monitored at least every 15 minutes. The temperature of the incubator should be monitored at least every minute. A summary of the water bath and/or incubator data should be included in the final report.

Humidity: Where applicable to the test, the relative humidity must be controlled within a tolerance of ±5% RH and recorded at least every 15 minutes.[6]

Light sensitivity: The active surface of some VVM models is sensitive to light. The laboratory should store and test these under "no-light" conditions.

5.2.3 Colour and OD measurements

For Test 2, colour measurements of the active surface (hue change evaluation) may be measured by a spectrodensitometer with a 2-mm diameter measurement area or alternatively the colour of "test patches" may be measured. Conversion of spectral data to optical density is defined within *ISO 5-3: 2009 Photography-Density measurements-Part 3: Spectral Conditions*. OD measurements for all relevant tests can be made using a densitometer with a 2-mm diameter measurement aperture with traceable calibration using BCRA[7] Series II tiles.

[5] The incubator temperature immediately around VVM samples can be made more stable by placing test samples inside a small ventilated glass or plastic box acting as a buffer. Validation of the lab method is essential.

[6] Humidity in the test chamber may be controlled accurately with salt solutions, e.g. for 33% RH - 370 g of Magnesium Chloride hexahydrate per 100 g of de-ionized water and for 75% RH - 45 g of Sodium Chloride per 100 g of de-ionized water or another method may be used that meets the requirement. These quantities may need to be adjusted to meet the required measured RH.

[7] BCRA = British Ceramic Research Association.

5.2.4 Reaction rates

Reaction rates are specific to different types of VVM, relating to groups of vaccines according to their heat stability at two specific temperature points. See Tables 1a and 1b.

Table 1a: VVM reaction rates by type

Type (Vaccines)	Maximum time to end point at +37°C	Maximum time to end point at +25°C	Maximum time to end point at +5°C	Time to end point at +5°C
VVM30: High stability	30 days	193 days	NA*	≥4 years
VVM14: Medium stability	14 days	90 days	NA*	≥ 3 years
VVM11: Intermediate stability	11 days	71 days	NA*	≥2.5 years
VVM7: Moderate stability	7 days	45 days	NA*	≥2 years
VVM2: Least Stable	2 days	NA*	225 days	NA*

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

Table 1b: VVM reaction rates by type

Type (Vaccines)	Maximum time to end point at +55°C	Maximum time to end point at +45°C	Maximum time to end point at 37°C (approx.)	Time to end point at +25°C
VVM250: Very High Stability	17 days	73 days	250 days*	≥900 days

**VVM (Arrhenius) reaction rates determined at 55°C and 45°C, the 37°C values are approximate*

Unless otherwise specified, the two temperatures and time periods highlighted in Tables 1a and 1b for each VVM type will be the agreed test period for testing each type.

Additionally, each VVM type 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

- **Step 1:** For each VVM type, select a random sample of 20 VVMs.
- **Step 2:** Measure the linear dimensions and calculate the areas of the active surface and reference surface with a suitable instrument, e.g. with a travelling microscope.

Acceptance / rejection criteria: The linear dimensions and the ratio of the areas of the inner active surface to the outer reference surface to comply with PQS Specification **E006/IN05.2** Annex 1.

5.2.6 Test 2: Characterising the colour change and shade change

- **Step 1:** Incubate three test patches or VVMs for each VVM type in Table 1a that must be tested at +37 ± 0.2°C in an incubator or a water bath. Incubate three test patches or VVMs for each VVM type in Table 1b that must be tested at +55 ± 0.2°C in an incubator or a water bath. In a water bath the VVMs must be inside sealed waterproof pouches to ensure no water contamination[8].
- **Step 2:** Measure the colour and optical density of the active surface positioning the instrument heads at the centre of the test patch or VVM. Also measure the colour and optical density of the reference surface in three different consistent places, e.g. 3 o'clock, 6 o'clock and 9 o'clock. Take readings at time zero and then repeat the reading at the same time each day for each of the three samples in each VVM type until the end point for that type of VVM is reached. All readings must be taken at room temperature and in the shortest possible time. Return test patches or VVMs immediately to the incubator or place back into sealed pouch(es) and returned to the water bath.
- **Step 3:** Plot the difference in L value[9] from the colour readings between the start point and that measurement that is read on each day against the corresponding change in optical density for the active surface. Report the delta-L versus delta-OD plot to the client. It is essential that these results are discussed with the client before proceeding with the remaining tests.

Acceptance / rejection criteria: To comply with PQS Specification **E006/IN05.3** Clause 4.2.2, there must be a shade change without a hue change. Any change in hue must be small enough so as to not significantly affect the validity of the densitometer OD readings which are used for the remainder of the tests.

[8] One method is to seal one waterproof pouch inside another waterproof pouch to ensure no water ingress (5-mil heat sealable foil polyethylene polyester MILB-131 Class 1 Type 1 envelopes may be used). It is up to the laboratory to prove that their method is watertight.

[9] The "L-value" from CIE Lab color space.

5.2.7 Test 3: Verifying the start point

Sample size: 20 for each VVM type.

Initial procedure:

- Measure the OD on the same portion of the active surface on all 20 VVM samples for each type.
- Take five samples at random in each type, measure the OD for three different portions of the reference surface, and calculate the average reading for each sample.

Acceptance / rejection 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 OD for all 20 samples must be within ±0.03 OD of the mean for that type.
- The difference between the active surface OD and the reference surface OD for each type must conform to the specification for the start point set out in the data sheet in the PQS Catalogue.
- The active surface OD measurements at the start point for each type should conform to the specification set out in in the data sheet in the PQS Catalogue.
- The reference surface OD measurements at the start point of each type should conform to the specification set out in in the data sheet in the PQS Catalogue.
- The three readings taken from the reference surface should conform to the specification for homogeneity set out in **E006/IN05.3**, Clause 4.2.3.
- The optical density of one portion of the reference surface compared with another portion of the reference surface should conform to the variation of the reference surface specification set out in **E006/IN05.3**, Clause 4.2.4.

If all the above conditions are met, the remaining 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 ofg
the samples will be used as described in Clauses 5.2.13 through 5.2.15. All activated samples must be stored at or below – 24°C until testing begins.

5.2.8 Test 4: VVM reaction rate +37°C, no light & +55°C, no light

+37°C, no light applies to all VVM types in Table 1a
+55°C, no light applies to all VVM types in Table 1b

Sample size: 60 VVMs for Set I.

- **Step 1:** Divide Set I into three subsets of 20 samples each. Condition sample Set I to +37 ± 0.2°C for VVM types in Table 1a or at +55 ± 0.2°C for VVM types in Table 1b in an incubator or in pouch in a water bath, without light, and at a humidity between 33%RH and 75%RH. Remove appropriate subset as indicated below and measure the reference surface and the active surface OD.
- **Step 2:** After 75% of the agreed test period, remove the first subset and measure the OD of the reference surface and the active surface on the 20 samples.
- **Step 3:** After completion of the agreed test period, remove the second subset and measure the reference surface and the active surface OD on the 20 samples.
- **Step 4:** After 125% of the agreed test period, remove the third subset and measure the reference surface and the active surface OD on the 20 samples.

Acceptance / rejection criteria: 95% of samples from Step 2 must have R-I values ≥0.00 OD[10]. 95% of samples from Step 3 must have R-I values ≤0.00 OD.

5.2.9 Test 5: VVM reaction rate +37°C, 75% RH, no light & +55°C, 75% RH, no light

+37°C, 75% RH, no light applies to all VVM types in Table 1a
+55°C, 75% RH, no light applies to all VVM types in Table 1b

Sample size: 60 VVMs for Set II.

- **Step 1:** Divide Set II into three subsets of 20 samples each. Condition VVM Set II (60 samples) to +37 ± 0.2°C for VVM types in Table 1a or at +55 ± 0.2°C for VVM types in Table 1b in an incubator, without light and at a relative humidity of 75 ± 5%. Remove appropriate subset as indicated below and measure the reference surface and the active surface OD.
- **Step 2:** After 75% of the agreed test period, remove the first subset and measure the reference surface and the active surface OD on the 20 samples.
- **Step 3:** After completion of the agreed test period, remove the second subset and measure the reference surface and the active surface OD on the 20 samples.

[10] See PQS Specification E006/IN05.3 Clause 4.2.6: Overall uncertainty (applies in Test 4 through Test 8)

- **Step 4:** After 125% of the agreed test period in the incubator, remove the third subset and measure the reference surface and the active surface OD on the 20 samples.

Acceptance / rejection acceptance criteria: 95% of samples from Step 2 must have R-I values ≥0.00 OD. 95% of samples from Step 3 must have R-I values ≤0.00 OD.

5.2.10 Test 6: VVM reaction rate +37°C, 33% RH, no light & +55°C, 33% RH, no light

+37°C, 33% RH, no light applies to all VVM types in Table 1a
+55°C, 33% RH, no light applies to all VVM types in Table 1b

Sample size: 60 VVMs for Set III.

- **Step 1:** Divide Set III into three subsets of 20 samples each. Condition VVM Set II (60 samples) to +37 ± 0.2°C for VVM types in Table 1a or at +55 ± 0.2°C for VVM types in Table 1b in an incubator, without light and at a relative humidity of 33 ± 5%. Remove appropriate subset as indicated below and measure the OD of the reference surface and the active surface.
- **Step 2:** After 75% of the agreed test period, remove the first subset and measure the OD of the reference surface and the active surface on the 20 samples.
- **Step 3:** After completion of the agreed test period, remove the second subset and measure the OD of the reference surface and the active surface on the 20 samples.
- **Step 4:** After 125% of the agreed test period in the incubator, remove the third subset and measure the OD of the reference surface and the active surface on the 20 samples.
- These Set III third subset samples should be stored at 5°C for 30 days in preparation for Clause 5.2.13, Reversion test.

Acceptance / rejection criteria: 95% of samples from Step 2 must have R-I values ≥0.00 OD. 95% of samples from Step 3 must have R-I values ≤0.00 OD.

5.2.11 Test 7: VVM reaction rate +25°C, no light & +45°C, no light

+25°C, no light applies to VVM30, VVM14, VVM11 and VVM7 types only
+45°C, no light applies to VVM types in Table 1b

Sample size: 60 VVMs for Set IV.

- **Step 1:** Divide Set IV into three subsets of 20 samples each. Condition VVM Set II (60 samples) to +25 ± 0.2°C without light in a water bath, or in an incubator at a relative humidity maintained between 33 ± 5% and 75 ± 5%. Remove appropriate subset as indicated below and measure the reference surface and the active surface OD.
- **Step 2:** After 60% of the agreed test period, remove the first subset and measure the OD of the reference surface and the active surface on the 20 samples.
- **Step 3:** After completion of the agreed test period, remove the second subset and measure the OD of the reference surface and the active surface on the 20 samples.

Acceptance / rejection criteria: 95% of samples from Step 2 must have R-I values ≥0.00 OD. 95% of samples from Step 3 must have R-I values ≤0.00 OD.

5.2.12 Test 8: VVM reaction rate +5°C, no light

+5°C, no light applies to VVM2 type only

Sample size: 60 VVMs for Set IV.

- **Step 1:** Divide Set IV into three subsets of 20 samples each. Condition VVM Set IV (60 samples) to +5 ± 0.2°C without light in a water bath, or in an incubator at a relative humidity maintained between 33 ± 5%RH and 75 ± 5%RH. Remove appropriate subset as indicated below and measure the reference surface and the active surface OD.
- **Step 2:** After 60% of the agreed test period, remove the first subset and measure the OD of the reference surface and the active surface on the 20 samples.
- **Step 3:** After completion of the agreed test period, remove the second subset and measure the OD of the reference surface and the active surface on the 20 samples.

Acceptance / rejection criteria: 95% of samples from Step 2 must have R-I values ≥0.00 OD. 95% of samples from Step 3 must have R-I values ≤0.00 OD.

5.2.13 Test 9: Reversion test

Applies to all VVM types

Sample size: 20 VVMs from the third subset of Set III.

- **Step 1:** As instructed in Clause 5.2.10 Step 4, store the third portion of 20 samples from Set III at +2°C to +8°C for 30 days.
- **Step 2:** Re-measure the active surface OD of the samples and compare the readings with those taken at the end of the Test 6.

Acceptance / rejection criteria: The active surface results must comply with PQS Specification **E006/IN05.3,** Clause 4.2.7 Reversion.

5.2.14 Test 10: Soak test

Applies to all VVM types

Sample size: 20 VVMs.

- **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 with no waterproof pouches at +2°C to +8°C for 8 hours. Seal the second (dry) group of labels in 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 +2°C to +8°C for 16 hours.
- **Step 5:** Remove both groups from the desiccant chamber and place in waterproof pouches in a water bath or incubator at +37 ± 0.2°C for VVM types in Table 1a or at +55 ± 0.2°C for VVM types in Table 1b. Once a day, remove the labels from the water bath and measure the active surface OD of each VVM until the end point is reached.

Acceptance / rejection criteria: The average results of the soaked versus the average results of the dry OD measurements should be compared for conformity to **E006/IN05.3**, Clause 4.2.8 (no more than a difference of 0.04 in optical density or ± 10% of initial (R – I) whichever is greater).

5.2.15 Test 11: Observer perception test

Applies to all VVM types

Sample size: 15 VVMs.

- **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 approximately 50% of the shade 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 at or below -24°C to prevent further shade change.
- **Step 3:** Evaluation. Five untrained observers, working independently under tungsten or fluorescent light at 100 lux on the working plane, must sort the vaccine vials into three groups (unchanged, 50% changed and end point). Record the level of the light used.

Acceptance / rejection 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:** Characterisation 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 surface – 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.

7. Prequalification evaluation

A product will qualify for inclusion on the register of PQS prequalified VVMs in accordance with WHO procedures provided the final report indicates full conformity with the requirements of specification **E006/IN05.3.**

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) in a timely manner. If any change is considered to adversely 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

10. Revision history

Revision history			
Date	Change summary	Reason for change	Approved
14 Mar 2006	Test procedure redrafted with general amendments to the form of words but not the content. Normative references, definitions and additional clauses added.	To achieve conformity with PQS documentation standards	UK
30 Nov 2006	General revisions	Following consultations with industry	UK (30 Nov 2006 – PQS Secretariat
07 Apr 2011	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 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	 Industry consultation Previous model no longer available Consistency Industry consultation Consistency	UK & AG
19 Jan 2012	References changed for consistency	E06 changed to E006	DM Jan 2012
22 May 2017	Test protocol amended to reflect the changes in the revised specification	For clarity	IG May 2017
22 May 2017	All sections of this specification have been completely revised	Consultation with industry and PQS team.	IG May 2017