VERIFICATION OF YELLOW FEVER ASSAYS

Purpose of verification

- Corroborate test performance in lab
- Assess potential for errors
- Inform implementation
- Meet regulatory guidelines

Requirements

- Time commitment
- Expense
- Careful design
- Use of available guidelines

Q&A

Q: What is assay verification?

A: Ensuring that when your lab runs the assay, it performs as per the manufacturer's claims and published performance criteria.

Q: How does assay verification differ from validation?

A: Verification involves limited testing to confirm assay performance whereas validation is a comprehensive performance evaluation.

Q: What happens if the assay fails verification?

A: Check adherence to protocol; repeat assay verification. Consult with Regional Coordinator upon repeat failure. Consider post-market surveillance reporting if relevant. Do not use assay until resolved.

Q: When is assay verification performed versus validation?

A: Assay verification is performed once in your lab for unmodified moderate and high complexity tests such as those requiring use of an instrument, approved by a stringent regulatory authority, and/or endorsed by WHO prior to routine use. Validation is only needed for laboratory-developed tests, off-label use of WHO-endorsed commercial assays, and commercial assays not yet endorsed by WHO.

Q: What does the result mean if the assay passes verification?

A: You r lab can consider integrating the assay into routine use as per recommended algorithms and guidelines.

COMPONENTS OF ASSAY VERIFICATION

Accuracy**.....** how closely do the results compare to the reference values?

Precision**.....** how closely do results for the same samples generated under different conditions agree with one another (e.g. 3 different operators, 3 different days)?

Reportable range**.....** range of values where test yields accurate results (quantitative or qualitative)

Reference range**.....** range of expected "normal" test outcomes for local population not affected by condition targeted by the assay as listed for intended use in manufacturer's instructions for use

SAMPLES USED FOR ASSAY VERIFICATION

Previously-tested non-haemolyzed reference samples, calibrators or well-preserved archived specimens with known values from your own or other labs

Minimum of 10 positives* and 10 negatives* *(avoid borderline samples)*

High positives (3-5) **Medium positives (3-5)** **Low positives (3-5)** **Negatives (10)**

* High positives can be diluted using true negative sample material to create medium and low positives

** The panel for Negatives ideally includes one or two non-cross-reactive sera which are positive for other disease(s) with clinically-similar presentation

DOCUMENTATION REQUIREMENTS

Purpose and Background – Methods – Results – Conclusions
Supervisor/manager and Laboratory Director approve and sign
Share with WHO
Save for > 2 years after test is discontinued

The Laboratory Supervisor or Quality Manager is responsible for assay verification

STEP BY STEP GUIDE

ASSAY VERIFICATION——STEP BY STEP GUIDE

PLANNING

1. Understand test principle, limitations, sample and control requirements, equipment needed.
2. Review manufacturer's performance data and identify which of accuracy, precision, reportable range and reference range are listed and calculation methods used for each e.g. Mean, SD, %CV. You will use the same methods.
3. Obtain enough tests (same lot number) and sufficient sample/control numbers and volume to perform entire assay verification.
4. Prepare protocol and working documents; schedule operators. Determine acceptance criteria (usually equal to manufacturer's reported performance for qualitative; CV<20% for semi/quantitative assays).

TIP
Manufacturer may not always list reportable and reference ranges

NOTE
Ensure equipment is calibrated and tests are not expired

NOTE
Assays are run without deviation from the IFU

PERFORM THE ASSAY VERIFICATION

1. For each assay, record kit lot number, date, and operator.
2. **Accuracy:** one operator tests all samples in a single run. Determine test validity and compare results to reference results. Compare accuracy to those reported by manufacturer.
3. **Precision:** Test all samples on three different days. Determine test validity and calculate precision. Compare to manufacturer's reported precision. *Note:* Accuracy component can be used in precision.
4. **Reportable range (usually quantitative assays):** Report the highest and lowest numerical value obtained from correct results.
5. **Reference range (some assays):** Report highest and lowest value for negative (normal) samples.

IDEALLY
Perform accuracy test in triplicate (intra-assay reproducibility). Ideally use different operators to assess precision

TIP
Use blind-coded samples so results can be used to document operator proficiency

POST-ASSAY VERIFICATION

Laboratory director signs SOP and assay verification (share with WHO); ensure reporting system can accommodate new assay results; integrate assay into laboratory workflow. Communicate assay verification outcome to relevant external parties.

REFERENCES

https://www.cms.gov/regulations-and-guidance/legislation/clia/downloads/6064bk.pdf
https://www.labce.com/spg2095637_assay_verification_and_validation.aspx
https://www.aacc.org/cln/articles/2014/july/bench-matters
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