Microbiologics®

Lab-Elite™ CERTIFIED REFERENCE MATERIAL

INTENDED USE

Lab-Elite™ Certified Reference Material (CRM) is a pure, homogenous, stable, preparation of lyophilized microorganism with well-characterized microscopic, macroscopic, phenotypic and genotypic characteristics. The identity of the CRM has been confirmed by sequencing of the ribosomal RNA (rRNA) gene. As CRM, this product is at zero passage.

A Certificate of Analysis, provided with the CRM, lists the phenotypic properties of the strain as well as the American Type Culture Collection (ATCC®) or other authentic reference culture number. A Sample Analysis Report and a Pulsed-Field Gel Electrophoresis (PFGE) report list the genotypic and phenotypic characteristics including rRNA based ID (identification) and DNA fingerprint of the organism with the stated restriction enzyme digest.

CRM is recommended for validation of a new method, for use in pharmacopeia compendial tests, and for quality control when a standard with known genotypic and phenotypic properties is required.

SUMMARY AND HISTORY

Microbiologics became ISO Guide 34 accredited in 2009 as a qualified reference material producer. ISO Guide 34 defines reference material as material that is "sufficiently homogeneous and stable with respect to one or more properties, which has been established to be fit for its intended use in a measurement process. Properties can be quantitative or qualitative (e.g. identity of substances or species)".[1]

Homogeneity of CRM is ensured by testing samples from each new lot for purity, viability and morphological characteristics. In order for the new lot of CRM to be released for sale, all samples must be pure, grow satisfactorily, and demonstrate morphological characteristics typical for the strain. Stability is monitored by testing the viability of each CRM lot at the end of its shelf life. Stability is deemed acceptable if good growth is obtained from the sample tested.

Lab-Elite™ Certified Reference Material is a lyophilized microorganism preparation. The use of this lyophilized material provides equivalent results to traditional methods used in preparing, storing and maintaining reference stock culture collections.

PRINCIPLE

Lab-Elite™ Certified Reference Material incorporates a lyophilization method, proposed by Obara et al., which uses a suspending medium consisting of gelatin, skim milk, ascorbic acid, dextrose, and charcoal[2]. The gelatin serves as a carrier for the microorganism. Skim milk, ascorbic acid, and dextrose protect the microorganism by preserving the integrity of the cell wall during freeze-drying and storage. The charcoal is included to neutralize any toxic substances formed during the lyophilization process.

FORMULA COMPONENTS

Each lyophilized preparation consists of:

- Microorganism population
- Gelatin
- Skim milk
- Ascorbic acid
- Dextrose
- Charcoal

PRODUCT DESCRIPTION

Lab-Elite™ Certified Reference Material is packaged inside a unit called a KWIK-STIK™. Each KWIK-STIK™ unit contains a lyophilized pellet of a single microorganism strain, a reservoir of hydrating fluid and an inoculating swab. The unit is sealed within a laminated pouch that contains a desiccant to prevent adverse moisture accumulation.

Lab-Elite™ Certified Reference Material is shipped in a canister containing one KWIK-STIK™ unit, a Certificate of Analysis, a Sample Analysis Report, a Pulsed-Field Gel Electrophoresis (PFGE) Subtyping Report and a product insert with instructions for use. The certificate and reports contain the following information:

1) Certificate of Analysis: Lists the microorganism name, catalog number, ATCC® or other authentic reference culture number, purity, recovery, expiration date, release information, macroscopic and microscopic features and phenotypic test results.
2) Sample Analysis Report: Reports the gram stain reaction, the genetic difference of the species in comparison to other closely related species, and the Microbial ID based on phenotypic and genotypic tests (rRNA gene).
3) PFGE Subtyping Report: Reports Restriction Fragment Length Polymorphism (RFLP) pattern associated with the strain using the stated restriction enzyme digest.

No mercury or latex is contained in the lyophilized preparation or KWIK-STIK unit.

PRECAUTIONS AND LIMITATIONS

- These products are for in-vitro use only.
- These devices, and subsequent growth of these microorganisms on culture media, are considered to be biohazard material.
- These devices contain viable microorganisms that may, under certain circumstances, produce disease. Proper techniques must be employed to avoid exposure and contact with any microorganism growth.
- The microbiology laboratory must be equipped, and have the facilities to receive, process, maintain, store and dispose of biohazard material.
- The microbiology laboratories personnel using these devices must be trained and able to demonstrate proficiency in processing, maintaining, storing and disposing of biohazard material.
- Agencies and statutes do regulate the disposal of all biohazard materials. Each laboratory must be aware of, and comply with, the proper disposal of biohazard materials.

STORAGE AND EXPIRATION

Store Lab-Elite™ Certified Reference Material at 2°C to 8°C in the original, sealed vial or pouch containing the desiccator. Stored as directed, the lyophilized microorganism preparation will retain its specifications and performance within the stated limits until the expiration date stated on the device label. The product should not be used if:

1) Stored improperly
2) There is evidence of excessive exposure to heat or moisture
3) The expiration date has passed

MATERIALS REQUIRED BUT NOT PROVIDED

The Technical Information Bulletin (TIB.081) *"Recommended Growth Requirements"* lists the recommended media and incubation requirements. This bulletin is available from our web site at www.microbiologics.com.

- Lab-Elite™ Certified Reference Material requires non-selective, nutrient or enriched agar media to optimize growth and recovery.
- Lab-Elite™ Certified Reference Material requires specific incubation times and conditions to optimize growth and recovery.

PRODUCT WARRANTY

These products are warranted to meet the specifications and performance printed and illustrated in product inserts, instructions, and supportive literature. The warranty, expressed or implied, is limited when:

- The procedures employed in the laboratory are contrary to printed and illustrated directions and instructions
- The products are employed for applications other than the intended use cited in product inserts, instructions, and supportive literature.

INSTRUCTIONS FOR USE OF CERTIFIED REFERENCE MATERIAL

Remove the Lab-Elite**™** (KWIK-STIK™) unit from 2°C to 8°C storage and allow the unopened pouch to equilibrate to room temperature.

1. Open the pouch and remove the KWIK-STIK™.
2. Tear off the pull tab portion of the label from the device. The label can be attached to permanent Quality Control Records or to the primary agar medium plate for identification.
3. Take note of the position of the pellet in the bottom part of the device and the reservoir of hydrating fluid in the top (cap) part of the device. Do **NOT** disassemble the device during hydration.
4. Release the hydrating fluid by breaking the ampoule using a pinching action at the middle of the ampoule in the cap of the device. Allow the hydrating fluid to flow through the swab shaft and **INTO** the bottom portion of the unit containing the lyophilized pellet.
5. Holding the device vertically, with the cap up, tap the bottom of the device on the counter to further facilitate the flow of the fluid.
6. Using a pinching action on the bottom portion of the unit, crush and mix the pellet in the fluid until the pellet particles are uniform in size and the suspension is homogenous in appearance.
7. **IMMEDIATELY,** saturate the swab with the hydrated material and transfer the material to an appropriate, non-selective, nutrient or enriched agar medium. With slight pressure, rotate the swab, and inoculate a circular area (i.e., one inch or 25 mm in diameter) of the agar medium. Using the same swab or a sterile loop, repeatedly (about 10 to 20 times) streak through the inoculated area and then continue to streak the remainder of the agar surface for isolation.
8. **IMMEDIATELY,** incubate the inoculated media at temperature and conditions appropriate to the microorganism.
9. Following the incubation, select representative well-isolated colonies for indicated transfers.
10. Discard any remaining hydrated material in accordance with the laboratory protocol for disposal of biohazardous materials.

TROUBLE SHOOTING GUIDE

If you are having a problem, refer to this guide and to the "*Recommended Growth Requirements*", TIB.081.

Problem	Possible Cause	Recommendations
No Growth	Improper storage	Refrigerate lyophilized microorganisms at 2-8°C upon arrival.
	Improper hydration	Break the ampoule at the top of the KWIK-STIK™ device. The liquid from the ampoule is used to emulsify the pellet. Do not incubate the hydrated suspension. Use hydrated pellet within thirty (30) minutes.
	Incorrect media	Follow the recommendations in the "*Recommended Growth Requirements*", TIB.081. Some microorganisms require special nutritive media. For example, anaerobes should be started on anaerobe media, Columbia Blood Agar or pre-reduced agar. It is best to start lyophilized microorganisms on nonselective agar.
	Incorrect temperature	Check the temperature in your incubator. Some microorganisms will not grow if the temperature is higher than 37°C. Verify that your thermometer is reading accurately. Perform incubator uniformity studies to rule out hot spots.
	Incorrect atmosphere	When anaerobic conditions are required, use an anaerobic indicator to ensure that anaerobic conditions are met.
	Too short an incubation period	A longer incubation period may be necessary. Some microorganisms, such as *Clostridium sporogenes*, can take 48 to 72 hours to grow.
Contamination	Incubation in broth	It is best to start lyophilized pellets on agar because contaminants multiply rapidly in broth.
Unexpected Test Results	Improper subbing	Do not perform phenotypic tests on pellet growth. Repeated sub-culturing may cause mutation. Always test fresh growth.
	Too large or small an inoculum	Follow the test manufacturer's instructions for inoculum size or density.
	Wrong QC microorganism	Use the Reference Culture recommended by the manufacturer or regulatory body.

QUALITY CONTROL

This product is developed, manufactured, and distributed:

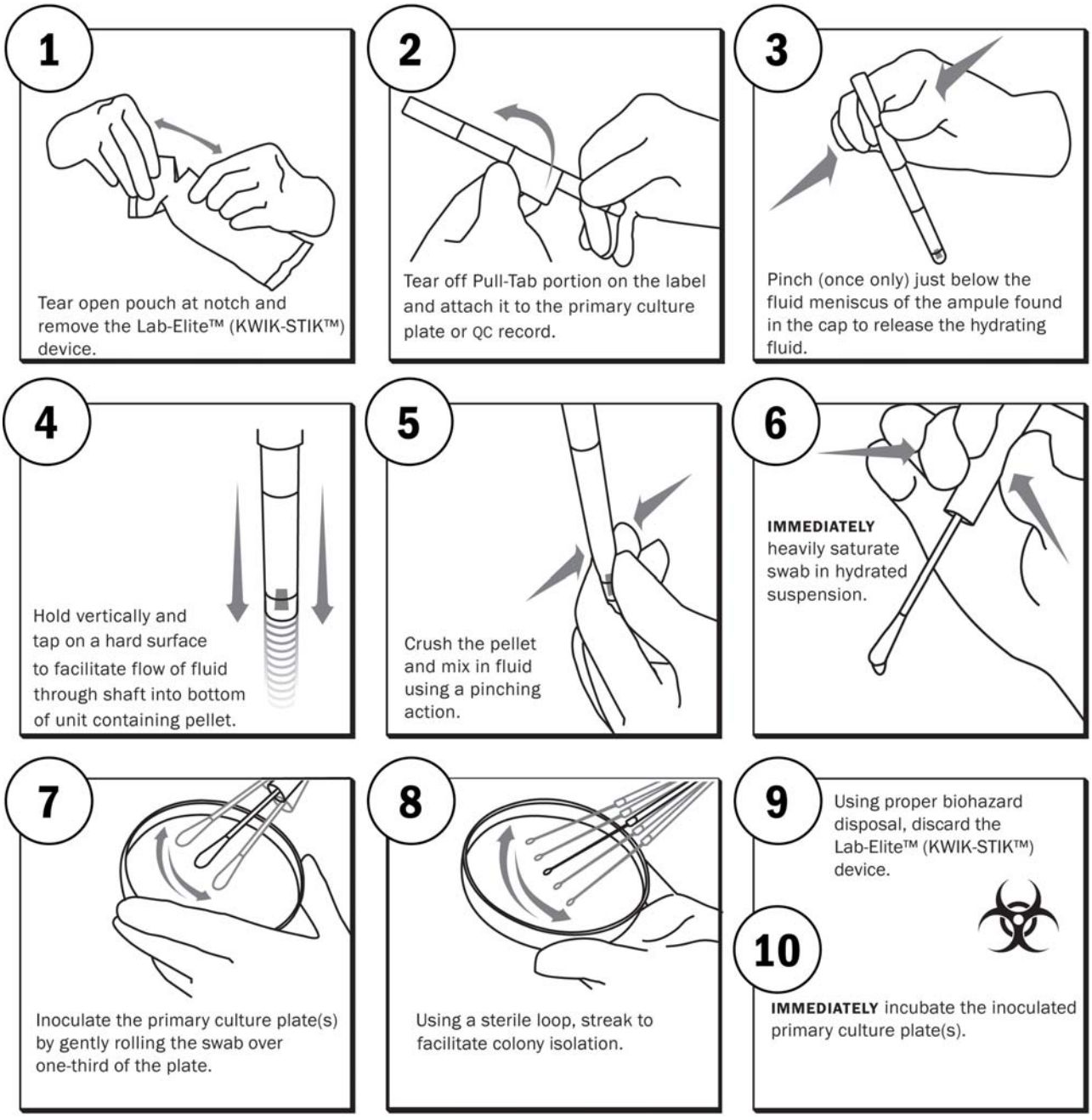
- In compliance with the mandates of FDA: Quality System Regulation (QSR), 21CFR Part 820
- In conformance with the elements of ISO 9001
- In conformance with CE Mark requirements
- In conformance with ISO Guide 34

Quality control functions may include, but are not limited to:

- Purity and growth characteristics
- Morphological features
- Molecular sub-typing by pulsed-field gel electrophoresis
- Biochemical activity
- The identity and traceability of the microorganism preparation to a reference culture

The decision to perform additional quality control is the responsibility of each individual laboratory.

ILLUSTRATED INSTRUCTIONS



BIOHAZARD CLEANUP

Should accidental leakage or spilling of the device or subsequent growth of the microorganism on agar media occur, the following information outlines materials and procedures which will safely facilitate the clean up of biohazard material.

1. Material Safety Data Sheet (MSDS)

- A file must be maintained with all MSDS documents for biohazard material.
- The MSDS file must be available to all employees.
- All employees must be made aware of the location of the MSDS files.

2. Biohazard Spill Kit

Biohazard Spill Kits are available from commercial sources or can be made with the following materials.

A. One liter bottle of an aqueous germicidal solution
B. One pair of disposable latex and/or latex free gloves
C. One tweezers
D. One Biohazard Bag with closure
E. One stack or roll of paper towels

3. Procedure

- Notify **ALL** people working in the immediate area of the incident.
- Do **NOT** leave the area unattended (unless you are the only individual in the area). Designate another employee to watch the incident area and divert traffic away from the incident area.
- After notifying all employees in the immediate area, collect the Biohazard Spill Kit and **IMMEDIATELY** return to the area.
- Put on the disposable gloves.
- With the tweezers, pick up as much material as possible and carefully place the materials into the Biohazard Bag.
- Saturate the spill area with germicidal solution.
- Keep the spill area moist with the germicidal solution for the appropriate amount of time as indicated on the germicidal solution used.
- Wipe up the area with the paper towels.
- Place all used paper towels in the Biohazard Bag.
- Following the cleanup, carefully remove the gloves and place into the Biohazard Bag.
- Seal the Biohazard Bag.
- Dispose of the Biohazard Bag in compliance with regulatory requirements.

KEY OF SYMBOLS

EC REP	Authorized Representative in the European Community	LOT	Batch Code (Lot)
	Biological Hazards and Risks		Caution consult accompanying documents Attention, see instructions for use
CE	CE Mark		Manufacturer
IVD	In Vitro Diagnostic Medical Device	REF	Catalog Number
	Temperature Limitation		Use by

WEB SITE

Visit our web site for current technical information and product availability **www.microbiologics.com**

REFERENCES

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ACKNOWLEDGEMENTS

1. ISO Guide 34:2009. International Organization for Standardization. 3rd Edition, 2009. Prepared by the ISO Reference Materials Committee
2. Y. Obara, S. Yamai, T. Nikkawa, Y. Shimoda, and Y. Miyamoto. 1981. J. Clin. Microbiol. 14:61-66.

The selection of reference stock cultures is only one integral part of the overall scheme for QC challenge procedures and techniques. Reference to guidelines for each laboratory's applications is essential. Examples might include:

1. Clinical Microbiology Procedures Handbook. ASM. Washington, D.C.
2. FDA Bacteriological Analytical Manual.
3. Manual of Clinical Microbiology, ASM, Washington, D.C.
4. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. CLSI.
5. Official Methods of Analysis of the Association of Official Analytical Chemists.
6. Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI.
7. Quality Assurance for Commercially Prepared Microbiological Culture Media. CLSI.
8. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. CLSI.
9. Standard Methods for the Examination of Dairy Products. American Public Health Association.
10. Standard Methods for the Examination of Water and Wastewater. American Water Works Association.
11. US Pharmacopeia and National Formulary