



Frequently Asked Questions

What is Certified Reference Material?

ISO Guide 34 defines reference material as material that is “sufficiently homogenous and stable with respect to one or more specified 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 Certified Reference Material is accompanied by a certificate that provides the value of the specified property.

Does Microbiologics sell Certified Reference Material?

Yes, Microbiologics sells a product called Lab-Elite™ Certified Reference Material (CRM). Lab-Elite™ is a pure, homogeneous, stable, lyophilized microorganism preparation with well characterized phenotypic and genotypic properties. It is accompanied by a certificate which provides values of qualitative properties.

Microbiologics became officially recognized as a Reference Material producer in 2009, when the company achieved ISO Guide 34:2000 Accreditation. To achieve Guide 34 accreditation, Microbiologics demonstrated its scientific and technical competence and met the stringent requirements for producing accurate and reliable data.

How does Microbiologics ensure homogeneity of the Certified Reference Material?

Microbiologics ensures homogeneity of Lab-Elite™ CRM by analyzing a representative number of randomly chosen units in accordance with acceptable statistical procedures.

Why use Lab-Elite?

1. Lab-Elite can be used for the following purposes:
2. Assuring the quality of test results. ISO Guide 17025 states, “The laboratory shall have quality control procedures for monitoring the validity of tests and calibrations undertaken. This monitoring shall be planned and reviewed and may include...regular use of certified reference material and/or internal quality control using secondary reference materials.”²
3. Validation of non-standard methods. ISO Guide 17025 states, “For new test and/or calibration methods, procedures should be developed prior to the tests and/or calibrations being performed and should contain... reference standards or reference materials required”³
4. Quality control when a standard with known genotypic properties is needed.
5. Traceability. Because the DNA fingerprint of this product is known, it can be distinguished from other strains of the same species found in a user’s laboratory or products.
6. Identification of strains used in pharmacopeia compendial tests. In regards to microbiological cultures, USP <1117> states, “the confirmation of identity for commonly used laboratory strains should ideally be done at the level of genotypic analysis (i.e., DNA finger printing, 16S rRNA gene sequencing, or PCR analysis using suitably validated probes)”⁴
7. Quality Control of Research

How are Lab-Elite strains identified?

A polyphasic approach is used on each CRM lot produced at Microbiologics. The species is identified by determining microscopic and macroscopic characteristics and analyzing metabolic activities using a VITEK® 2 system. MEI, Molecular Epidemiology, Inc., re-authenticates the identity using ribosomal RNA (rRNA) gene sequencing and pulsed-field gel electrophoresis (PFGE). Gene sequencing identifies the

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species. Because there are many strains within a species, PFGE is used to subtype the microorganism strain.

How is Lab-Elite packaged?

Lab-Elite packaging is identical to the Microbiologics® KWIK-STIK™. Each Lab-Elite device contains a lyophilized pellet of a single microorganism strain, a reservoir of hydrating fluid and an inoculating swab. The entire Lab-Elite kit is packaged inside a canister which includes the following: one Lab-Elite CRM device, a Certificate of Analysis, a Sample Analysis Report, a PFGE Sub-typing Report and a product insert.

What information do the Lab-Elite reports provide?

1. Certificate of Analysis:
 - Microscopic and macroscopic characteristics.
 - Biochemical results obtained on the Vitek 2.
2. Sample Analysis Report:
 - A microscopic picture of the microorganism.
 - A genetic identification based on ribosomal RNA (rRNA) gene sequencing.
 - Comparisons to genetically similar microorganisms based on genetic distance.
3. PFGE Subtyping Report:
 - The identification of the restriction enzymes used in the pulsed-field gel electrophoresis.
 - Photographs of the Restriction Fragment Length Polymorphism (RFLP) patterns.

How is gene sequencing performed on Lab-Elite products?

The 16S DNA gene is sequenced using 16S ribosomal RNA. Sequencing determines the order of the nucleotide bases – adenine, guanine, cytosine and thymine in a molecule of DNA. rDNA sequencing involves the following steps:

1. Lysis of the microorganism to release genomic DNA.
2. PCR amplification of the target DNA.
3. Sequencing reaction to incorporate dye-tagged nucleotides.
4. DNA sequence determination using an automated capillary electrophoresis DNA sequencer system.
5. Sequence comparison against a reference ribosomal sequence database.

What does genetic distance mean on the Sample Analysis Report?

Genetic distance refers to the genetic divergence between species. Smaller genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic relationship.

How is PFGE performed on the Lab-Elite products?

PFGE is pulsed-field gel electrophoresis. It is used to establish relatedness among organisms belonging to the same species or, in the case of Lab-Elite, to confirm the identity of a strain. PFGE uses the following steps to obtain a DNA fingerprint of the Lab-Elite microorganism strain:

- A suspension of bacteria cells is made.
- The cells are lysed to release intact DNA.

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- The DNA is digested using a restriction enzyme. This causes the breakup of the DNA molecules into fragments. Fragment size varies depending on the microorganism strain.
- A tracking dye is added to the sample.
- A pulsed electrical field forces the fragments to travel through an agarose gel by subjecting the fragments to different voltages from varying angles at different times. Populations of molecules migrate through the gel at a speed related to their size.
- A Restriction Fragment Length Polymorphism (RFLP) banding pattern forms on the agarose gel. Each Lab-Elite microorganism strain forms a unique RFLP pattern.

The restriction enzymes used in the analysis of the CRM are stated on the PFGE Report.

What strains are available in the Lab-Elite product line?

Lab-Elite is offered in the microorganism strains listed below. Additional strains will be added as requested.

Catalog Number	Description
0998-CRM	<i>Bacillus cereus</i> ATCC®10876™*
0318-CRM	<i>Clostridium perfringens</i> ATCC®13124™*
0366-CRM	<i>Enterococcus faecalis</i> ATCC®29212™*
0483-CRM	<i>Escherichia coli</i> ATCC®8739™*
0335-CRM	<i>Escherichia coli</i> ATCC®25922™*
0484-CRM	<i>Pseudomonas aeruginosa</i> ATCC®9027™*
0353-CRM	<i>Pseudomonas aeruginosa</i> ATCC®27853™*
0485-CRM	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC®6538™*
0360-CRM	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC®25923™*
0827-CRM	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC®6538P™*
0371-CRM	<i>Staphylococcus epidermidis</i> ATCC®12228™*
0385-CRM	<i>Streptococcus pyogenes</i> ATCC®19615™*

References

¹ISO GUIDE 34. Third edition 2009. General requirements for the competence of reference material producers. 3.4

²ANSI/ISO/IEC 17025-2005. American National Standard. General requirements for the competence of testing and calibration laboratories. 5.9.1

³ANSI/ISO/IEC 17025-2005, American National Standard. General requirements for the competence of testing and calibration laboratories. 5.4.5.2

⁴The United States Pharmacopeia. 2010. <1117> Microbiological Best Laboratory Practices

Acknowledgements



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