SECTION

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WHY DO WE IDENTIFY?

In the pharmaceutical, healthcare and cosmetic manufacturing industries we identify and classify micro-organisms for a number of reasons:

- To help us gather information that could lead to determining the source of the contamination and therefore allow us to take appropriate preventative action to avoid or minimise future recurrence;
- To help us to understand the normal microbial flora that is inherent to the raw materials used to manufacture our products, or that are present in our manufacturing environments;
- To help us understand the trends of microbial contamination in the materials used to manufacture our products;
- To help to determine the microbiological risk to the product and/or the patient or consumer;
- To build up a library of organisms for culture media testing and method validation.

There are different levels of identification we can carry out and how far we go will depend on:

- Where the micro-organism was isolated from,
- The levels of the micro-organism recovered,
- Pharmacopoeia requirements,
- The criticality of the identification.

Depending on these factors and what decisions are to be made using the final identification, a presumptive (genus level) or confirmed (species level) identification of the microorganism can be made.

This document will provide you with an overview of bacterial identification – the why's, the when's and the how's. It will begin with the basics of taxonomy and bacterial nomenclature to provide you with the background on how bacteria are classified and named. A brief history of bacterial identification will be given, leading into a summary of the identification methods available to us as microbiologists, with the advantages and limitations of different methods discussed. Considerations for rapid identification methods, choosing an appropriate method for your laboratory, and qualifying the method of choice will also be discussed. Note, this document covers commonly used methods within the pharmaceutical, healthcare and cosmetic manufacturing industries, and is not all encompassing. Other systems and alternative methods are available.

Microbial Identification

Identification is the determination of where a microorganism sits within a classification scheme, and ultimately results in assigning it a name.

Taxonomy is a branch of science for organising microorganisms into groups or taxa based on similar morphological, physiological and/or genetic characteristics and naming them accordingly, which forms the basis for further identification. A hierarchical classification system generally consists of the following taxa in which all micro-organisms are classified (as per Table 1):

Table 1: Bacterial classification

Scientific Classification	Example
Domain	Bacteria
Kingdom	Eubacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	*Staphylococcus*
Species	*Staphylococcus aureus*

A genus contains different species that have several features in common. Placement of a species into a particular genus is based on a number of shared phenotypic and genotypic characteristics.

A species is the most basic of the taxonomic groups and is defined as a collection of bacterial strains that share common physiological and genetic features, and differ significantly from other microbial species. In some species groups, subgroups can be designated that share some specific but relatively minor characteristics. These subgroups can be called subspecies, serotypes, biotypes or genotypes.

Micro-organisms are named using a binomial (two name) system. Every organism is assigned a genus and a species name, and generally these are of either of Latin or Greek origin. Naming of micro-organisms is done using established rules and guidance set out in the International Code of Nomenclature of Bacteria (ICNB) or the Bacteriological Code (BC). The first letter of the genus designation is always capitalised and the species name is always given lower case. When written down, both names are written simultaneously and italicised or underlined. For example, the *Staphylococci* include *Staphylococcus aureus*. Advances in metagenomics are leading to new patterns of DNA relatedness and changes to taxonomy, where group strains are updated on the basis of overall genetic similarity.

History of Microbial Identification

In the early days of microbial identification, Microbiologists relied on simple phenotypic data such as the micro-organisms colony morphology and cell morphology to classify isolates.

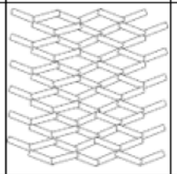
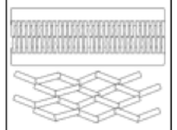
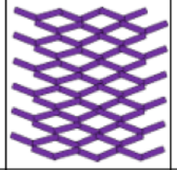
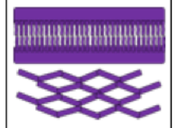
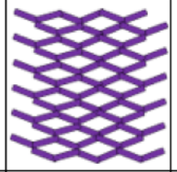
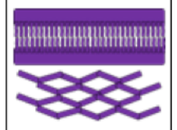
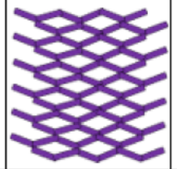
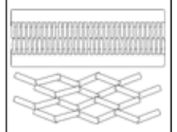
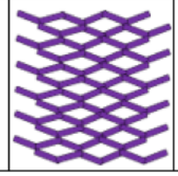
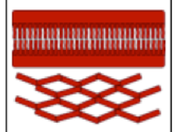
A notable milestone in the history of microbial identification was in 1884 when Dr Hans Christian Gram developed a stain to make bacterial cells more visible under the microscope. It has since been used to differentiate bacteria based on their cell wall structure. This method proved very useful in characterising micro-organisms into Gram-positive and Gram-negative, but is difficult to perform correctly. The method is subjective in how long it takes to decolourise making it difficult to always get reliable results (one industry wide survey put the annual error rate at up to 3%)[9]. The Gram stain is also dependent on how old the culture is and how it was grown (an 18-24 hour culture is recommended, although this will be dependent on the rate of growth).

Most identification methods today, even the most advanced systems, still rely on the Gram stain to direct the choice of system so it is important to get it right and be aware of these pitfalls.

The reader should note that approaches to Gram-staining vary in terms of times and the use of counter stains. The above is diagram is illustrative.

Today, a wide variety of identification methods are available. These can largely be split into three groups:

Figure 1: Outlining the Gram-stain (Image: Creative Commons)

Gram Staining Procedure		Gram Positive Cell Wall		Gram Negative Cell Wall	
Process of test	Appearance of Cells	Effect of Step	Effect on Cell Wall	Effect of Step	Effect on Cell Wall
Step 1: Begin with heat fixed cells		**Step 1:** Cell wall remains clear.		**Step 1:** Cell wall remains clear.	
Step 2: Flood slide with crystal violet dye for 1 min.		**Step 2:** Peptidoglycan cell wall is flooded with crystal violet and appears purple.		**Step 2:** Cell wall is stained purple from the crystal violet dye.	
Step 3: Add iodine solution for 1 min.		**Step 3:** A crystal violet – iodine complex is formed within the peptidoglycan cell wall trapping the purple stain.		**Step 3:** A crystal violet-iodine complex is formed but does not adhere to the cell wall due to the thin layer of peptidoglycan.	
Step 4: Wash slide with alcohol for 20sec.		**Step 4:** The crystal violet – iodine complex is trapped with the peptidoglycan cell wall and doesn't wash out.		**Step 4:** The crystal violet – iodine structure is washed out of the thin peptidoglycan layer.	
Step 5: Counter stain with safranin.		**Step 5:** As the peptidoglycan cell wall remains stained purple the red safranin has no effect.		**Step 5:** The red safranin stains the washed gram negative cells.	

- **Phenotypic methods** are based on morphological, physiological and/or biochemical features expressed by the organism during growth.
- **Chemotaxonomic methods** are based on the chemical constituents of the cell, for example antigen content, cell wall composition, cellular fatty acid content or whole cell protein content.
- **Genotypic methods** are based on the genetic makeup of the organism, for example its nucleic acids.

There is no single universal method available that is capable of identifying all micro-organisms and so a polyphasic approach using a number of methods is often used, particularly where a definitive identity is required. Modern bacterial taxonomy uses several methods from each of the groups to characterise new microorganisms thoroughly in order to classify and eventually give them a name. This means that distinguishing key features of each micro-organism group are readily available to us as industrial microbiologists and therefore we can look for these key features to identify our isolates rather than use a cumbersome polyphasic approach for each and every isolate we recover. However, it is important to be mindful that not all methods are 100% accurate 100% of the time and we need to make sure we gather enough data around the isolate to be confident that the identification is correct, regardless of the method we choose.