MICROBIOLOGY DIAGNOSTICS

Comprehensive Guide to bioMérieux API 20E for Microbial Identification: Technical Principles, Clinical Protocols, and Analytical Interpretation

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In the field of clinical and industrial microbiology, the precise identification of Gram-negative bacilli is a cornerstone of diagnostic accuracy, epidemiological surveillance, and therapeutic efficacy. For over five decades, the **Analytical Profile Index (API)**, specifically the **API 20E** system developed by bioMérieux, has served as a global gold standard for the identification of **Enterobacteriaceae**and other non-fastidious Gram-negative rods. This miniaturized biochemical panel revolutionized laboratory workflows by replacing large volumes of test tubes with a streamlined, standardized, and digitally integrated methodology.

The Evolution of Microbial Identification: The API 20E Framework

The API 20E system is a standardized identification system that utilizes 21 miniaturized biochemical tests and a robust database to identify bacterial species. This methodology is based on the traditional principles of phenotypic characterization, where the metabolic activity of a microorganism is assessed through its interaction with specific substrates. By miniaturizing these tests into a single plastic strip (gallery), bioMérieux enabled laboratories to achieve high-throughput results with minimal reagent waste and reduced physical footprint.

The importance of the API 20E strip extends beyond its physical design; it is supported by the **apiweb™**platform, a comprehensive digital knowledge base that translates biochemical reaction patterns into definitive taxonomic identifications. This synergy between biochemical hardware and bioinformatics software ensures that even atypical strains of common pathogens are identified with high statistical confidence.

Technical Architecture of the API 20E Strip

The API 20E strip consists of 20 microtubes containing dehydrated substrates. These substrates are reconstituted by adding a bacterial suspension, which initiates metabolic processes during an incubation period (typically 18 to 24 hours). The reactions are manifested as color changes, either spontaneously or following the addition of specific reagents.

The 20 Biochemical Tests Explained

The strip is divided into several metabolic categories, including carbohydrate fermentation, amino acid decarboxylation, and miscellaneous enzymatic activities. Below is a detailed breakdown of the primary tests included in the API 20E gallery:

- ONPG: Tests for the presence of the enzyme β-galactosidase using the substrate ortho-nitrophenyl-βD-galactopyranoside. A positive result is indicated by a yellow color change.
- ADH, LDC, ODC: These tests measure the decarboxylation of Arginine, Lysine, and Ornithine, respectively. These reactions occur under anaerobic conditions, often requiring the addition of mineral oil to create an airtight seal in the microtube cupule.
- CIT: Evaluates the ability of the organism to utilize citrate as its sole carbon source.
- H2S: Detects the production of hydrogen sulfide through the reduction of sodium thiosulfate.

- URE: Tests for urease activity, which releases ammonia and raises the pH, changing the indicator to pink/red.
- TDA: Tryptophan Deaminase activity, visualized by adding ferric chloride.
- IND: Indole production from tryptophan, visualized with Kovac’s reagent.
- VP: The Voges-Proskauer test for the production of acetoin (acetylmethylcarbinol).
- GEL: Detects the liquefaction of gelatin by proteolytic enzymes.
- Carbohydrate Fermentation (GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY, ARA): These wells test the organism’s ability to ferment specific sugars, resulting in acid production that lowers the pH and changes the color of the phenol red indicator from blue-green to yellow.

Standard Operating Procedure: Practical Implementation

Successful identification using the API 20E system requires strict adherence to standardized protocols. Deviations in inoculum density or incubation temperature can lead to false-negative or ambiguous results.

Step 1: Inoculum Preparation

The process begins with the preparation of a pure culture on a suitable primary isolation medium (e.g., MacConkey agar or Blood Agar). A single, well-isolated colony is picked and suspended in 5 mL of sterile saline (0.85% NaCl) or API Suspension Medium. The turbidity must be adjusted to match the **0.5 McFarland standard**to ensure a consistent bacterial concentration (approximately 1.5 x 10^8 CFU/mL).

Step 2: Inoculation of the Gallery

Using a sterile pipette, the suspension is distributed into the microtubes. For specific tests (ADH, LDC, ODC, H2S, URE), the cupule section of the microtube must be filled with sterile mineral oil to create anaerobic conditions. For other tests (like CIT, VP, and GEL), both the tube and the cupule are filled with the bacterial suspension.

Step 3: Incubation and Reading

The gallery is placed in an incubation box with a small amount of water added to the honeycombed bottom to maintain a humid environment, preventing the dehydration of the microtubes. The system is incubated at 36°C (± 2°C) for 18 to 24 hours. After incubation, the results are recorded. Some tests (TDA, IND, VP) require the addition of reagents before they can be read.

Test	Reagent Required	Positive Reaction	Negative Reaction
TDA	TDA Reagent (Ferric Chloride)	Dark Brown / Reddish-Brown	Yellow
IND	JAMES Reagent (Kovac’s)	Pink / Red Ring	Yellow / Pale Green
VP	VP 1 & VP 2 (KOH & Alpha-Naphthol)	Pink / Red (after 10 mins)	Colorless / Pale Yellow
GLU (Oxidation)	None	Yellow (fermentation) / Blue (Oxidation)	Blue-Green

Data Analysis: The Numerical Profile Index

One of the most innovative aspects of the API 20E system is the conversion of biochemical results into a numerical profile. This 7-digit (or 9-digit) code allows for precise identification through the apiweb™ database.

Calculating the Profile Number

The 20 tests are divided into groups of three. Within each group, a value is assigned to a positive reaction based on its position: 1 for the first test, 2 for the second, and 4 for the third. A negative reaction is always assigned a value of 0. The sum of these values creates a single digit for each group.

For example, if in the first group (ONPG, ADH, LDC):- ONPG is (+) = 1- ADH is (-) = 0- LDC is (+) = 4The first digit of the profile would be **1 + 0 + 4 = 5**.

This process is repeated for all seven groups, resulting in a 7-digit profile (e.g., 5144572). This profile is then entered into the **apiweb™**software, which compares the profile against thousands of entries in the database to identify the species and provide a percentage of certainty.

Comparison of Microbial Identification Methods

While API 20E remains a staple, it is essential to understand its position relative to other diagnostic technologies. The following table provides a comparative analysis of API 20E, manual tube methods, and automated systems like VITEK® 2.

Feature	Manual Tube Methods	API 20E Strip	Automated Systems (VITEK 2)
Setup Time	High (Manual Prep)	Medium	Low (Automated)
Turnaround Time	24-48 Hours	18-24 Hours	5-12 Hours
Standardization	Low (Operator Dependent)	High (Standardized Reagents)	Very High
Database Support	Limited / Manual Books	Extensive (apiweb™)	Real-time Cloud Database
Cost per Test	Low	Moderate	High (Requires Equipment)
Required Expertise	Very High	Moderate	Low/Moderate

Applications in Clinical and Industrial Microbiology

The versatility of the API 20E makes it applicable in diverse settings. In clinical microbiology, it is primarily used for identifying pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella*species from patient samples. In the food industry, it is utilized to detect contaminants and ensure the safety of production batches.

Identification of Enterobacteriaceae

The Enterobacteriaceae family contains numerous human pathogens. API 20E is specifically optimized for these organisms. For instance, differentiating between *Salmonella* and *Proteus* can be achieved through the H2S and Urease tests. *Salmonella* is typically H2S positive and Urease negative, while *Proteus* is frequently Urease positive.

The Role of API 20E in E-Katalog Procurement

In regions like Indonesia, the procurement of diagnostic kits such as the **BIOMERIEUX API 20 E (Ref: 20100)** is often managed through government procurement platforms like **E-Katalog 5.0 (LKPP)**. This ensures that healthcare facilities have access to standardized, high-quality diagnostic tools at regulated prices, facilitating consistent laboratory standards across the national health system.

Troubleshooting and Limitations

Despite its robustness, the API 20E system is not without challenges. Understanding potential failure modes is critical for senior laboratory personnel.

Common Troubleshooting Scenarios

- Weak Reactions: If a reaction color is borderline (e.g., pale orange instead of yellow in sugar tests), it should generally be considered negative unless the manufacturer’s instructions suggest otherwise. Using a slightly higher inoculum density may resolve this in repeat testing.
- False Negatives in ADH/LDC/ODC: These tests are highly sensitive to oxygen. If the mineral oil overlay is insufficient, the resulting oxidation may mask the decarboxylation reaction, leading to an incorrect profile.
- Contamination: If the primary culture was not pure, the API profile will likely be "unacceptable" or "doubtful." Always perform a purity plate alongside the API strip.
- Non-Fermenters: If an organism is a non-fermenter (e.g., Pseudomonas), the carbohydrate tests will remain negative (blue-green). In such cases, the API 20 NE (Non-Enterobacteriaceae) strip is the more appropriate tool.

The Future of Phenotypic Identification

As genomic sequencing (WGS) and Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry become more prevalent, some have questioned the longevity of biochemical strips. However, the API 20E remains relevant for several reasons:

1. Accessibility: Small to medium-sized labs may not have the capital to invest in MALDI-TOF mass spectrometers or NGS platforms.
2. Phenotypic Confirmation: Understanding the actual metabolic behavior (phenotype) of a bacterium is often necessary to confirm genomic predictions.
3. Standardization: The API database is one of the most rigorously curated sets of phenotypic data in existence, making it an essential reference even for high-tech facilities.

In conclusion, the bioMérieux API 20E system represents a pinnacle of diagnostic engineering that balances complexity with usability. By integrating miniaturized biochemistry with advanced digital interpretation, it provides microbiologists with a reliable, standardized path to bacterial identification. Whether used in a clinical setting to guide patient therapy or in an industrial lab to safeguard public health, the API 20E continues to be an indispensable tool in the microbiologist’s arsenal, bridging the gap between traditional techniques and the digital future of diagnostics.

Baca Juga (Artikel Terkait)

- **The Comprehensive Guide to Bacterial Identification: In-Depth Biochemical Testing Protocols and Diagnostic Frameworks**
  URL: http://alghafgolden.com/comprehensive-guide-bacterial-identification-biochemical-tests.pdf
- **The Definitive Guide to API 20E: Advanced Microorganism Identification and Biochemical Profiling in Modern Microbiology**
  URL: http://alghafgolden.com/api-20e-microorganism-identification-biochemical-profiling.pdf
- **A Comprehensive Technical Guide to the API® 20E and API® Microbial Identification Systems**
  URL: http://alghafgolden.com/comprehensive-guide-api-20e-microbial-identification.pdf

Tags: #bioMérieux #API 20E #Microbial Identification #Clinical Laboratory #Enterobacteriaceae #Biochemical Testing