MICROBIOLOGY DIAGNOSTICS

A Comprehensive Technical Guide to the API® 20E and API® Microbial Identification Systems

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In the field of clinical and industrial microbiology, the ability to accurately identify bacterial species is paramount for diagnosis, treatment planning, and quality control. One of the most significant advancements in this domain was the development of the **Analytical Profile Index (API)**. Pioneered by bioMérieux, the API system transitioned traditional, labor-intensive biochemical testing into a miniaturized, standardized, and highly reproducible format. This article provides an in-depth technical exploration of the API 20E system, its biochemical foundations, procedural workflows, and its broader application in microbial diagnostics.

The Evolution of Microbial Identification: From Tubes to Strips

Before the advent of miniaturized systems, microbial identification relied on the 'macromethod,' which involved inoculating a series of test tubes filled with different biochemical media. This method was not only time-consuming but also prone to variability due to differences in media preparation, inoculum size, and interpretation across different laboratories. The introduction of the **API® 20E**revolutionized this process by consolidating 21 miniaturized biochemical tests onto a single plastic strip.

The API 20E is specifically designed for the identification of **Enterobacteriaceae**and other non-fastidious, Gram-negative rods. By utilizing a standardized database and numerical profile system, the API platform allows for species-level identification within 18 to 24 hours, significantly reducing the turnaround time for critical diagnostic information.

Core Concepts and Theoretical Framework

The API system operates on the principle of biochemical phenotyping. Each microorganism possesses a unique set of enzymes and metabolic pathways that allow it to utilize specific substrates. When a bacterial suspension is added to the dehydrated substrates in the API strip, metabolic activities occur, resulting in color changes that can be visually interpreted or measured spectrophotometrically.

The Miniaturized Cupule Structure

Each API strip consists of 20 microtubes containing dehydrated substrates. These microtubes are composed of a **tube** (the lower part containing the substrate) and a **cupule**(the upper aerated section). This design allows for two distinct environments within a single strip:

- Anaerobic Conditions: Achieved by filling the tube and then overlaying the cupule with sterile mineral oil.
- Aerobic Conditions: Achieved by filling only the tube and leaving the cupule open to the atmosphere.

Biochemical Reaction Categories

The tests on an API 20E strip can be categorized into several functional groups:

- Carbohydrate Fermentation/Oxidation: Determining the ability of the organism to produce acid from specific sugars (e.g., Glucose, Arabinose, Sorbitol).
- Enzymatic Activity: Detecting the presence of specific enzymes such as Urease, Gelatinase, and Beta-galactosidase (ONPG).

- Decarboxylation and Deamination: Measuring the organism's ability to metabolize amino acids like Lysine, Arginine, and Ornithine.
- Miscellaneous Metabolic Tests: Including Citrate utilization, H_2S production, and Indole production.

Technical Analysis of the API 20E Test Battery

To understand the precision of the API 20E, one must analyze the specific biochemical reactions occurring within the strip. The following table provides a technical breakdown of the primary tests included in the API 20E system.

Test Mnemonic	Substrate / Active Ingredient	Reaction Detected	Positive Color Result
ONPG	2-nitrophenyl-βD-galactopyranoside	Beta-galactosidase activity	Yellow
ADH	L-arginine	Arginine dihydrolase activity	Red/Orange
LDC	L-lysine	Lysine decarboxylase activity	Red/Orange
ODC	L-ornithine	Ornithine decarboxylase activity	Red/Orange
CIT	Trisodium citrate	Citrate utilization	Turquoise/Blue
H2S	Sodium thiosulfate	H2S production	Black deposit/film
URE	Urea	Urease activity	Red/Orange
TDA	L-tryptophane	Tryptophane deaminase activity	Dark brown
IND	L-tryptophane (Reaction with James reagent)	Indole production	Pink/Red ring
VP	Sodium pyruvate (Reaction with KOH/Alpha-naphthol)	Acetoin production (Voges-Proskauer)	Pink/Red
GEL	Kohn's gelatin	Gelatinase activity	Black pigment diffusion
GLU	D-glucose	Fermentation/Oxidation	Yellow/Grayish

The Role of Indicators

Most tests in the API 20E strip utilize pH indicators to signal metabolic changes. For carbohydrate fermentation tests, **bromothymol blue**is commonly used. When an organism ferments a sugar, it produces organic acids, lowering the pH and shifting the indicator from blue/green to yellow. Conversely, in decarboxylation tests, the removal of a carboxyl group from an amino acid produces alkaline amines, raising the pH and shifting the indicator toward red or violet.

Standard Operating Procedure: Practical Implementation

For high-accuracy results, adherence to the **Standard Operating Procedure (SOP)**is critical. The procedure involves several phases: preparation, inoculation, incubation, and reading.

1. Inoculum Preparation

The accuracy of the API 20E is highly dependent on the concentration of the bacterial suspension. A pure culture must be grown on an isolation medium (e.g., Blood Agar or MacConkey Agar). A single, well-isolated colony is picked and suspended in 5 ml of 0.85% NaCl (sterile saline) or sterile water. The turbidity must be adjusted to match a **0.5 McFarland standard**to ensure a consistent number of bacteria per milliliter (approximately 1.5 x 10^8

CFU/ml).

2. Inoculating the Strip

Using a sterile pipette, the suspension is distributed into the tubes of the strip. Specific instructions must be followed for cupule management:

- For tests ADH, LDC, ODC, H2S, and URE, the tube is filled and the cupule is topped with mineral oil to create an anaerobic environment.
- For the CIT, VP, and GEL tests, both the tube and the cupule are filled with the suspension.
- For all other tests, only the tube is filled.

3. Incubation

The strip is placed in an incubation box containing a small amount of water to maintain humidity. It is then incubated at **34-37°C for 18 to 24 hours**. For certain non-enteric organisms (like Pseudomonas), longer incubation or lower temperatures (30°C) may be required.

4. Reagent Addition and Reading

After the incubation period, certain tests require the addition of external reagents to visualize the result:

- TDA Test: Add 1 drop of TDA reagent.
- IND Test: Add 1 drop of JAMES reagent.
- VP Test: Add 1 drop of VP1 and 1 drop of VP2 reagents; wait 10 minutes.
- Oxidase Test: This is performed separately from the strip and the result is recorded in the 21st slot of the results sheet.

The Numerical Profile and Data Management

The API 20E system uses a unique method for data interpretation known as the **Numerical Profile**. The 21 tests (20 on the strip + oxidase) are divided into groups of three. Each test in a group is assigned a value if positive:

1. First test in group: 1
2. Second test in group: 2
3. Third test in group: 4

If a test is negative, its value is 0. By summing the values within each group, a **7-digit profile number** is generated. For example, if all three tests in the first group are positive, the first digit of the profile is 7 (1+2+4). If only the first and third are positive, the digit is 5 (1+0+4).

This 7-digit code is then looked up in the **API Profile Index** or entered into the **apiweb™** software. This database contains thousands of profiles for different species, providing a statistical likelihood of the identification (e.g., "Excellent Identification to Escherichia coli").

Comparative Analysis: API 20E vs. API 20NE

While the API 20E is the flagship product for Enterobacteriaceae, bioMérieux offers specialized strips for other groups. The **API 20NE** is designed for non-fastidious, non-enteric Gram-negative rods, such as Pseudomonas and Acinetobacter.

Feature	API 20E	API 20NE
Target Organisms	Enterobacteriaceae & Gram-neg rods	Non-enteric Gram-negative rods
Number of Tests	21 (including Oxidase)	20 (including Oxidase/NO2)
Incubation Time	18-24 Hours	24-48 Hours
Metabolic Focus	Fermentation & Enzyme activity	Oxidation & Assimilation
Substrates	Sugar fermentation, Decarboxylases	Hydrolysis, Assimilation of carbon sources

Troubleshooting and Reliability Factors

Despite its robustness, several factors can lead to inaccurate results in the API system. Understanding these failure modes is essential for senior laboratory personnel.

Labile Tests: ODC and ARA

Research has shown that the **Ornithine Decarboxylase (ODC)** and **Arabinose (ARA)**tests are among the most labile on the API 20E strip. These tests are sensitive to changes in inoculum density and incubation environment. If a quality control strain (like Proteus mirabilis ATCC 35659) fails to produce the expected results for these tests, the entire batch of strips must be scrutinized.

Common Procedural Errors

- Inoculum Concentration: A suspension that is too thin may lead to false negatives (weak reactions), while a suspension that is too thick can cause false positives or ambiguous color shifts.
- Improper Oil Overlay: Failing to add mineral oil to the ADH, LDC, ODC, H2S, and URE cupules will introduce oxygen, preventing the anaerobic reactions required for these tests and leading to incorrect profiles.
- Media Carryover: Using colonies from highly selective media (like XLD or SS agar) can sometimes carry over inhibitory chemicals into the suspension, affecting the biochemical reactions. It is best to use non-selective agar for the final inoculum preparation.

Case Study: Identifying an Atypical Salmonella

In a clinical setting, an isolate showing typical Salmonella morphology on Hektoen Enteric agar was tested using API 20E. The initial 7-digit profile was ambiguous. Upon review, the H2S reaction was weakly positive, and the lysine decarboxylase (LDC) was positive, but the citrate (CIT) was negative. By extending the incubation to 48 hours and re-checking the profile, the system correctly identified the isolate as a specific subspecies of Salmonella enterica. This case highlights the importance of combining visual morphology with API data and, when necessary, supplemental testing.

Broader Implications in Biomanufacturing and Global Health

The API system's influence extends far beyond the clinical microbiology lab. In **biomanufacturing**, maintaining a sterile environment is critical. API strips are used for environmental monitoring to identify contaminants in cleanrooms and production lines. The standardization provided by the API 20E allows pharmaceutical companies to comply with regulatory requirements (such as those from the FDA or EMA) for microbial identification and traceability.

Furthermore, in resource-limited settings, the API system provides a sophisticated diagnostic capability without the need for expensive automated machinery (like VITEK or MALDI-TOF MS). Its portability and lack of requirement for complex electronic infrastructure make it a staple in global health initiatives and mobile diagnostic laboratories.

As we look toward the future, the API system continues to evolve. While automated systems and molecular methods (like 16S rRNA sequencing) are becoming more common, the API's reliability, ease of use, and extensive database ensure it remains a gold standard for manual microbial identification. It serves as both a primary diagnostic tool and a vital confirmatory method in polyphasic taxonomy, bridging the gap between traditional microbiology and modern genomic approaches. The legacy of the API system is a testament to the power of standardized, miniaturized biochemistry in advancing our understanding of the microbial world.

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
- **Comprehensive Guide to bioMérieux API 20E for Microbial Identification: Technical Principles, Clinical Protocols, and Analytical Interpretation**
  URL: http://alghafgolden.com/api-20e-microorganism-identification-technical-guide.pdf

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