Unknown Bacteria “F” Lab Report

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Introduction

Laboratory testing plays a significant role in identifying microorganisms because different bacterial species can appear similar under ordinary observation while producing distinct staining, growth, and biochemical reactions. Correct identification is important in clinical microbiology, food safety, environmental testing, and research because the organism’s identity influences the interpretation of disease, contamination, and possible treatment. The original investigation involved an unknown bacterium labeled “F” and used Gram staining together with Eosin Methylene Blue agar, MacConkey agar, Mannitol Salt Agar, and Salmonella–Shigella agar. These methods generated a recognizable pattern: unknown F was a Gram-negative rod, produced colorless colonies on MacConkey and EMB media, did not visibly ferment mannitol, and formed colorless or transparent colonies with a black center on SS agar. (Centers for Disease Control and Prevention, n.d.)

The original report concluded that unknown F was *Salmonella Typhi*. The observations make *Salmonella* a reasonable presumptive possibility, especially because black-centered colonies on SS agar can indicate hydrogen-sulfide production. However, the available tests cannot distinguish *S. Typhi* reliably from other *Salmonella* serovars or from some hydrogen-sulfide-producing members of Enterobacterales, including certain *Proteus* and *Citrobacter* strains. The expanded report therefore preserves every recorded result while changing the level of certainty: unknown F is presumptively consistent with a non-lactose-fermenting, hydrogen-sulfide-producing enteric bacterium, with *Salmonella* among the leading possibilities. Confirmatory biochemical, serological, or molecular testing would be required before naming the organism *Salmonella Typhi*.

Purpose

The purpose of this laboratory report is to identify unknown bacterium “F” as far as the available evidence permits by examining its Gram reaction, cellular shape, growth on selective media, and fermentation or hydrogen-sulfide characteristics. The exercise also aims to explain what each test contributes to the identification process. A scientifically responsible unknown-organism report should not simply match one colony color with one species name. It should combine independent observations, consider alternative organisms, identify conflicting results, and distinguish between presumptive and confirmed identification. This approach preserves the original objective while making the reasoning more transparent.

The investigation also demonstrates why microbiologists use several media rather than one. Gram staining places an organism into a broad structural group. MacConkey and EMB agar help determine

whether a Gram-negative organism ferments lactose. SS agar selects for enteric pathogens and differentiates hydrogen-sulfide producers. Mannitol Salt Agar is designed mainly for salt-tolerant organisms such as staphylococci and therefore serves less clearly in the identification of a Gram-negative enteric isolate. Together, these results create a profile that guides the selection of follow-up tests.

Observation/Result

Different laboratory tests were performed on unknown F to determine its characteristics. The culture was streaked onto EMB, MacConkey, MSA, and SS agar plates, and a Gram stain was prepared from the culture. The most distinctive observation occurred on Salmonella–Shigella agar, where the colonies were reported as colorless and transparent with black centers. The blackening suggests production of hydrogen sulfide, which reacts with iron compounds in the medium to form a dark precipitate. The colorless background suggests that the organism did not ferment lactose under the test conditions.

The Gram stain showed pink or red rod-shaped cells, supporting classification as a Gram-negative bacillus. On MacConkey agar, smooth colorless colonies were observed, indicating non-lactose fermentation. On EMB agar, the culture grew as colorless colonies without the dark coloration or metallic green sheen associated with strong lactose-fermenting coliforms. On Mannitol Salt Agar, the original report described deep-red colonies and interpreted the result as failure to ferment mannitol. This result should be treated cautiously because the high salt concentration of MSA normally inhibits many Gram-negative enteric bacteria. The growth may reflect limited salt tolerance, contamination, mixed culture, or interpretation of weak growth and should be repeated with appropriate control organisms.

Gram Stain

The Gram stain differentiates bacteria according to properties of the cell envelope. Crystal violet first stains the cells, iodine forms a dye complex, alcohol or another decolorizer removes the complex from Gram-negative cells, and safranin counterstains those cells pink or red. Gram-positive bacteria retain the purple complex because of their thick peptidoglycan layer. The original reagent list contained crystal violet, iodine, decolorizer, and “afranin,” which should be corrected to safranin. Proper technique is essential because over-decolorization can make Gram-positive cells appear negative, while under-decolorization can make Gram-negative cells appear falsely positive.

Unknown F was reported as a Gram-negative rod. This result is compatible with *Salmonella*, but it is not specific. Many enteric bacteria, including *Escherichia*, *Shigella*, *Proteus*, *Citrobacter*, *Klebsiella*, and *Enterobacter*, share this appearance. The stain should ideally be repeated from a single isolated colony to confirm culture purity. If more than one cell shape or Gram reaction appears, the culture

may contain multiple organisms, making the plate results unreliable as one identification profile.

Mannitol Salt Agar

Mannitol Salt Agar contains a high concentration of sodium chloride and is designed primarily to select salt-tolerant staphylococci. It also contains mannitol and phenol red. An organism that ferments mannitol produces acid, changing the medium toward yellow, while a non-fermenter leaves it red or pink. The original observation that the colonies or surrounding medium remained deep red supports a negative mannitol-fermentation reaction if genuine growth occurred.

However, this medium is not a standard primary identification test for *Salmonella*. Many Gram-negative enteric organisms should grow poorly or not at all because of the salt concentration. The MSA result therefore does not confirm *S. Typhi*. Instead, it introduces a quality-control question. The culture should be restreaked for purity, Gram stained from the MSA colony, and compared with positive and negative control organisms. A good report includes inconsistent evidence rather than ignoring it.

MacConkey Agar

MacConkey agar is selective for many Gram-negative enteric bacteria because bile salts and crystal violet inhibit much Gram-positive growth. It is differential because lactose and the neutral-red indicator distinguish lactose fermenters from non-fermenters. Strong lactose-fermenting colonies typically become pink or red, whereas non-lactose fermenters remain pale or colorless. Unknown F formed smooth colorless colonies, supporting a non-lactose-fermenting phenotype.

This observation narrows the possibilities but does not identify one organism. Many typical *E. coli*, *Klebsiella*, and *Enterobacter* strains ferment lactose, so they become less likely. *Salmonella*, *Shigella*, *Proteus*, *Morganella*, *Providencia*, and some *Citrobacter* isolates can remain colorless. The MacConkey result should therefore be combined with hydrogen-sulfide production, urease, motility, citrate, indole, and other biochemical characteristics.

Eosin Methylene Blue Agar

EMB agar also favors the growth of many Gram-negative organisms and differentiates lactose fermentation. Eosin Y and methylene blue inhibit much Gram-positive growth and respond to acid produced during fermentation. Strong lactose fermenters may form dark colonies or a metallic green sheen, while weak or non-fermenters remain lightly colored or colorless. Unknown F produced growth with colorless colonies, agreeing with the MacConkey result.

Agreement between EMB and MacConkey strengthens the conclusion that unknown F was not a

strong lactose fermenter. The original report described EMB as a medium used to distinguish coliforms and fecal coliforms associated with water contamination. That is one important use, but the plate in this exercise functions more generally as part of a differential profile. Colorless growth is compatible with *Salmonella*, but it cannot distinguish the species or serovar.

Salmonella–Shigella Agar

SS agar is selective and differential for enteric organisms, especially suspicious *Salmonella* and *Shigella* colonies. It contains lactose, bile salts and other selective agents, a pH indicator, and compounds that reveal hydrogen-sulfide production. Lactose fermenters typically appear pink or red, whereas non-lactose fermenters appear colorless. Hydrogen sulfide reacts with ferric ions to create black centers or blackening.

The original report's observation of colorless, transparent colonies with black centers is the strongest evidence supporting presumptive *Salmonella*. *Shigella* generally does not produce hydrogen sulfide and would usually remain colorless without a black center. Nevertheless, black-centered colonies are not exclusive to *Salmonella*; certain *Proteus* and *Citrobacter* strains may produce a similar appearance. A suspicious colony should be picked, purified, and subjected to confirmation rather than being reported immediately as *S. Typhi*.

Interpretation of the Combined Results

The recorded results form a coherent partial profile. Gram-negative rod morphology indicates an enteric or another Gram-negative bacillus. Colorless colonies on MacConkey and EMB indicate absent or limited lactose fermentation. Black-centered colonies on SS agar indicate hydrogen-sulfide production. This combination makes *Salmonella* plausible and makes typical lactose-fermenting *E. coli* less likely. It also makes *Shigella* less likely because of the black-center result.

The remaining differential diagnosis includes *Proteus* and some *Citrobacter* strains. *Proteus* may show swarming on nonselective media and is usually strongly urease positive. Many *Salmonella* strains are urease negative. Citrate, indole, lysine decarboxylase, motility, and triple sugar iron reactions would further separate the candidates. The MSA growth should be repeated because it does not fit cleanly with the expected behavior of a Gram-negative enteric isolate.

Additional Tests Required for Confirmation

Triple Sugar Iron agar would help determine glucose, lactose or sucrose fermentation, gas formation, and hydrogen-sulfide production. A presumptive *Salmonella* isolate often produces an alkaline slant, acid butt, and hydrogen sulfide, although exact reactions vary. Lysine Iron Agar can test lysine decarboxylation and H_2S production. A urease-negative result would support *Salmonella* over *Proteus*,

while oxidase testing would help place the organism among Enterobacterales. Citrate, indole, and motility tests would provide additional differentiation.

Even a biochemical pattern consistent with *Salmonella* would not prove serovar Typhi. Serological testing for somatic and flagellar antigens, a validated molecular assay, MALDI-TOF mass spectrometry with appropriate confirmation, or reference-laboratory identification would be needed. Because *S. Typhi* is a human-restricted pathogen associated with enteric fever and public-health response, the serovar should not be assigned from colony appearance alone. (World Health Organization, n.d.)

Clinical Significance

Salmonella enterica serovar Typhi causes typhoid fever and spreads primarily through food or water contaminated with fecal material from an infected person or chronic carrier. Illness may include prolonged fever, headache, abdominal symptoms, weakness, and serious complications such as intestinal bleeding or perforation. Blood culture is an important diagnostic method because bacteremia commonly occurs. A student culture exercise, however, cannot establish clinical infection or guide individual treatment. (U.S. Food and Drug Administration, n.d.)

The original report listed ciprofloxacin and ceftriaxone as common treatment choices. Treatment now requires current susceptibility information because antimicrobial-resistant and extensively drug-resistant Typhi strains have emerged in several regions, including Pakistan. Antibiotics should therefore not be recommended solely from a presumed plate identification. The scientifically appropriate statement is that confirmed clinical isolates require susceptibility testing and management according to current medical and public-health guidance.

Sources of Experimental Error

Possible errors include mixed culture, incorrect plate labeling, over- or under-decolorization during Gram staining, heavy inoculation, expired media, unsuitable incubation time, and reading the plates after differential reactions became obscured. Carrying organisms between plates without sterilizing the loop could also create contamination. Positive and negative controls should be used to verify that the media and staining reagents perform as expected.

Recording colony size, shape, elevation, margin, texture, color, and the amount of growth would improve reproducibility. Photographs taken at the standard reading time could support interpretation. Repeating the tests from a well-isolated colony would determine whether the unusual MSA result belonged to the same bacterium as the black-centered SS colony.

Conclusion

The original laboratory results show that unknown bacterium F was a Gram-negative rod, did not ferment lactose strongly on MacConkey or EMB agar, and formed colorless colonies with black centers on SS agar. These observations are consistent with a hydrogen-sulfide-producing, non-lactose-fermenting enteric organism and support a presumptive identification of *Salmonella*. They do not provide enough evidence to conclude specifically that the organism is *Salmonella Typhi*.

The MSA result should be repeated because strong growth on this high-salt medium is unexpected for many enteric Gram-negative bacteria. Confirmation would require a pure culture, biochemical testing such as TSI, LIA, urease, citrate, indole, oxidase, and motility, followed by serological or molecular identification where appropriate. The final result should therefore be recorded as presumptive *Salmonella* or another H_2S-producing member of Enterobacterales, pending confirmatory testing. This conclusion preserves the original experimental observations while matching the certainty to the evidence actually obtained.

Works Cited

Centers for Disease Control and Prevention. “Clinical Guidance for Typhoid Fever and Paratyphoid Fever.” 2024.

U.S. Food and Drug Administration. *Bacteriological Analytical Manual, Chapter 5: Salmonella*. 2026.

World Health Organization. “Typhoid.” 2023.