



Laboratory Gram Stain Misidentifications of *Neisseria meningitidis* and the Impact on Public Health Response to Meningococcal Disease

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Background

Invasive meningococcal disease (IMD) caused by *Neisseria meningitidis*, a Gram-negative diplococci, can quickly lead to death or significant sequelae in survivors. *Neisseria meningitidis* transmission occurs by person-to-person contact and secondary cases can occur. Public health response to IMD cases includes identifying close contacts and administering post-exposure prophylaxis as soon as possible. Identification of Gram-negative diplococci provides initial evidence for an IMD diagnosis. California laboratories are required to immediately notify public health authorities about suspect IMD cases so public health control measures can be implemented. However, Gram stain interpretation is subjective and the accuracy of results may be affected by laboratorian training and experience. Initial misidentified Gram stain results are correctly identified when Gram stain is reviewed by a second laboratorian or when culture is confirmed. We determined the number of Gram stain misidentifications among confirmed IMD cases and assessed the impact of misidentification on public health response to these cases.

Objective

1. Identify Gram stain misidentifications among meningococcal disease cases.
2. Determine impact of meningococcal disease Gram stain misidentifications on public health response.

Methods

Study Design:

- Reviewed preliminary and final Gram stain results for IMD cases reported to California Department of Public Health during January 2013 through February 2015.
- Classified Gram stain search results as defined in Table 1.
- Assessed impact of Gram stain misidentifications on public health response to IMD cases by calculating the amount of time that elapsed between issuance of incorrect and corrected Gram stain reports.

Study Numbers:

- 179 IMD cases
- Records reviewed for 162 (91%) cases
- Awaiting 17 medical records/lab reports
- Included 159 cases with blood and 97 cases with CSF results
- Excluded three cases with specimen sources other than blood and CSF

Table 1. Definitions of Gram Stain Search Result Classifications

| |
|---|
| Correct: Initially correctly identified as Gram negative diplococci |
| Misidentified: Initially NOT identified as Gram negative diplococci THEN correctly identified as Gram negative diplococci |
| Inconclusive: No Gram stain results (Gram stain not performed or results not available) |
| Negative: No organisms seen on Gram stain |
| N/A: 1: Gram stain performed if growth indicated in culture 2: Lumbar puncture not performed |

Results

Figure 1. Classification of Blood Gram Stains
See Table 1 for definitions

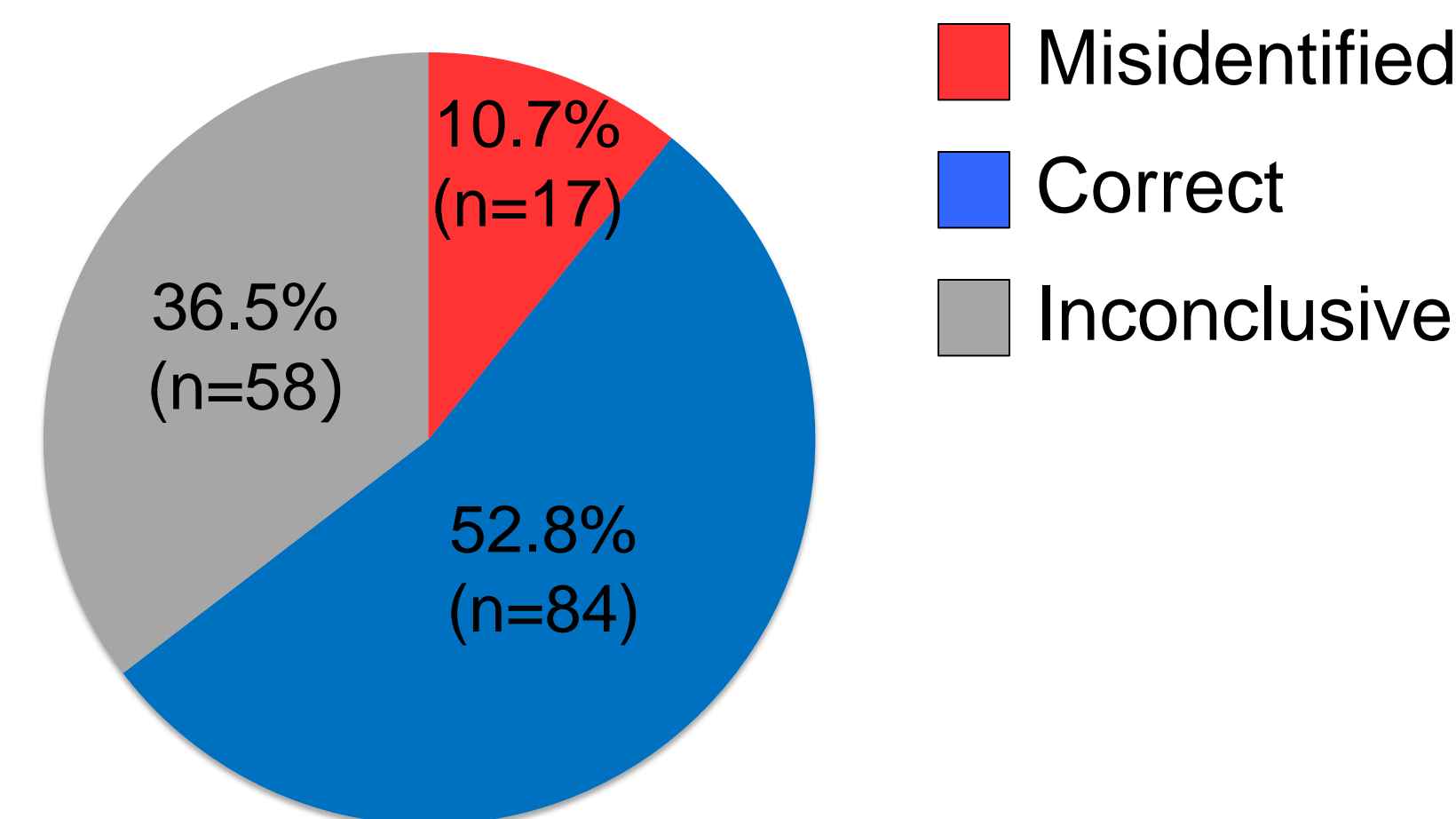


Figure 2. Classification of CSF Gram Stains
See Table 1 for definitions

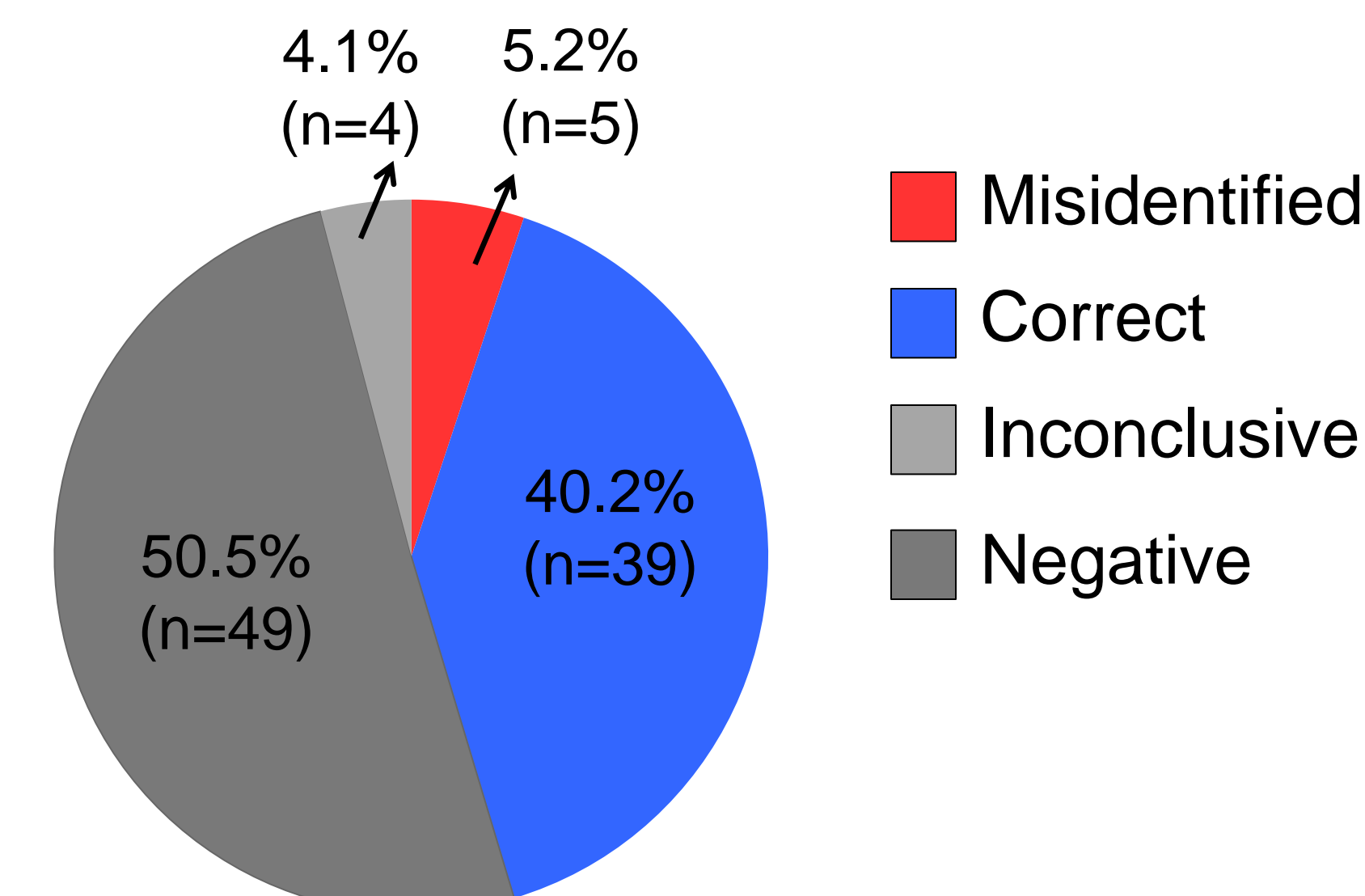


Table 2. Classification of Gram Stain Search Results

| Specimen Type | Number of Cases | Misidentified | Correct | Inconclusive | Negative |
|---------------|-----------------|---------------|---------|--------------|------------------|
| Blood | 159 | 17 (11%) | 84 | 58 | N/A ¹ |
| CSF | 97 | 5 (5%) | 39 | 4 | 49* |

* 6 cases were culture negative and PCR positive; 29 cases were culture negative

Table 3. Classification of CSF Gram Stain Results for Cases with Blood Gram Stain Results

| Blood Gram Stain Results | CSF Gram Stain Results | | | | |
|--------------------------------|------------------------|---------|--------------|----------|------------------|
| | Misidentified | Correct | Inconclusive | Negative | N/A ² |
| Misidentified (n=17) | 0 | 5 | 0 | 3 | 9 |
| Correct (n=84) | 2 | 18 | 1 | 25 | 38 |

Figure 3. Protocol for Reporting Gram Stain Results

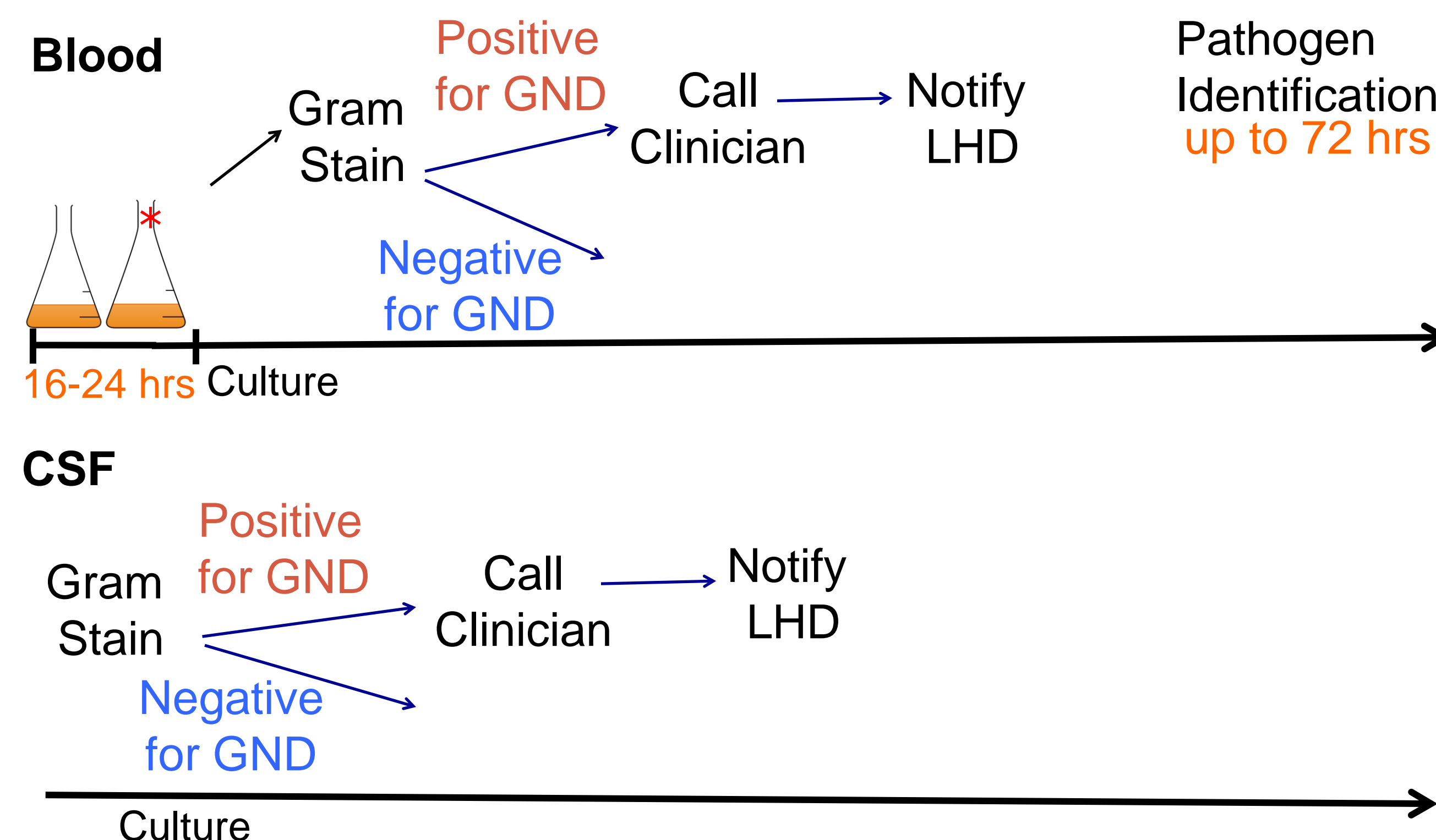


Figure 4. Public Health Impact of Gram Stain Misidentifications

| Initial Call | Corrected Call |
|--------------------------------------|----------------|
| Approximate notification delay (hrs) | |
| Case 1 | 47 |
| Case 2 | 37 |
| Case 3 | 18 |
| Case 4 | 18 |
| Case 5 | 15 |
| Case 6 | 12 |
| Case 7 | 12 |
| Case 8 | 11 |
| Case 9 | 1.5 |

Summary

- 14% of cases had at least one misidentified Gram stain result.
- 9% of cases had only a misidentified Gram stain result; cultures were positive for *Neisseria meningitidis*.
- The majority of Gram stain misidentifications occurred in blood specimens.
- The median delay in reporting suspect IMD cases to public health due to incorrect initial Gram stain results was 15 hours.

Conclusions

- Initial misidentification of *Neisseria meningitidis* Gram stains can occur and results in delayed reporting of IMD cases to public health.
- Delayed reporting leads to a delay in identification of contacts and administration of post-exposure prophylaxis.
- Misidentification could also result in exposure of laboratorians to *Neisseria meningitidis*.

Future Direction

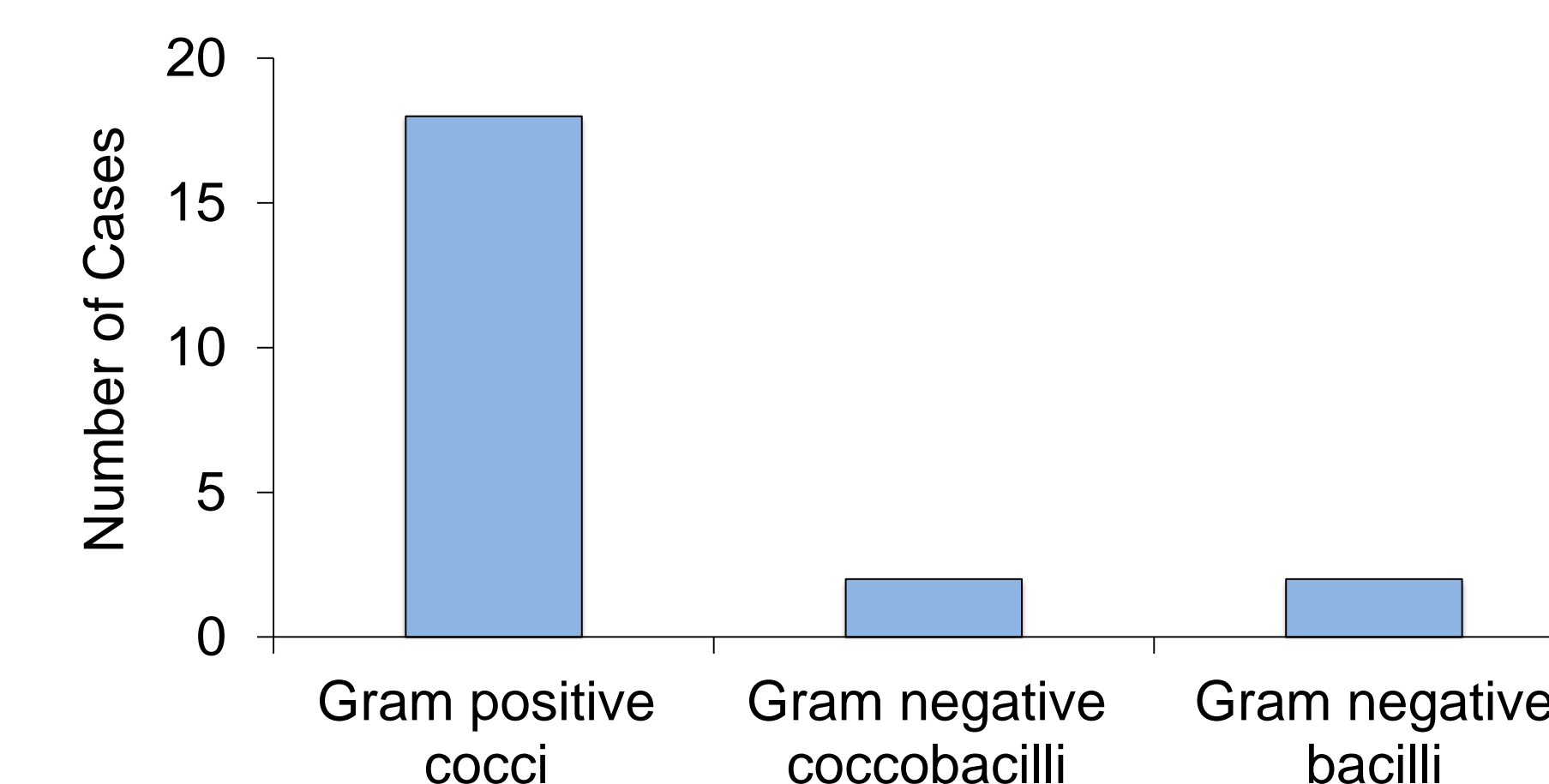
- Determine if Gram stain misidentifications are associated with level of experience of those performing and reading Gram stains.
- Determine protocols used by laboratories in performing, reading and reporting Gram stain to reduce the number of Gram stain misidentifications.
- Determine if lab personnel are at risk for exposure to *Neisseria meningitidis* because of failing to properly identify organism as Gram negative diplococci.

References

1. Rand KH, Tillan M. Errors in interpretation of Gram stains from positive blood cultures. Am J Clin Pathol 2006; 126:686-90.
2. Thairu Y, Nasir IA, Usman Y. Laboratory perspective of gram staining and its significance in investigations of infectious diseases. Sub-Saharan Afr J Med 2014; 1: 168-74.
3. Title 17, California Code of Regulations Reportable Diseases and Conditions

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Figure 5. Organisms Seen in Misidentified Gram Stains



Acknowledgments

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