# Evaluating a Fully Automated 16S/tNGS Solution for the Detection of Bacterial and Fungal Pathogens in Formalin-fixed, Paraffin-embedded (FFPE) tissues
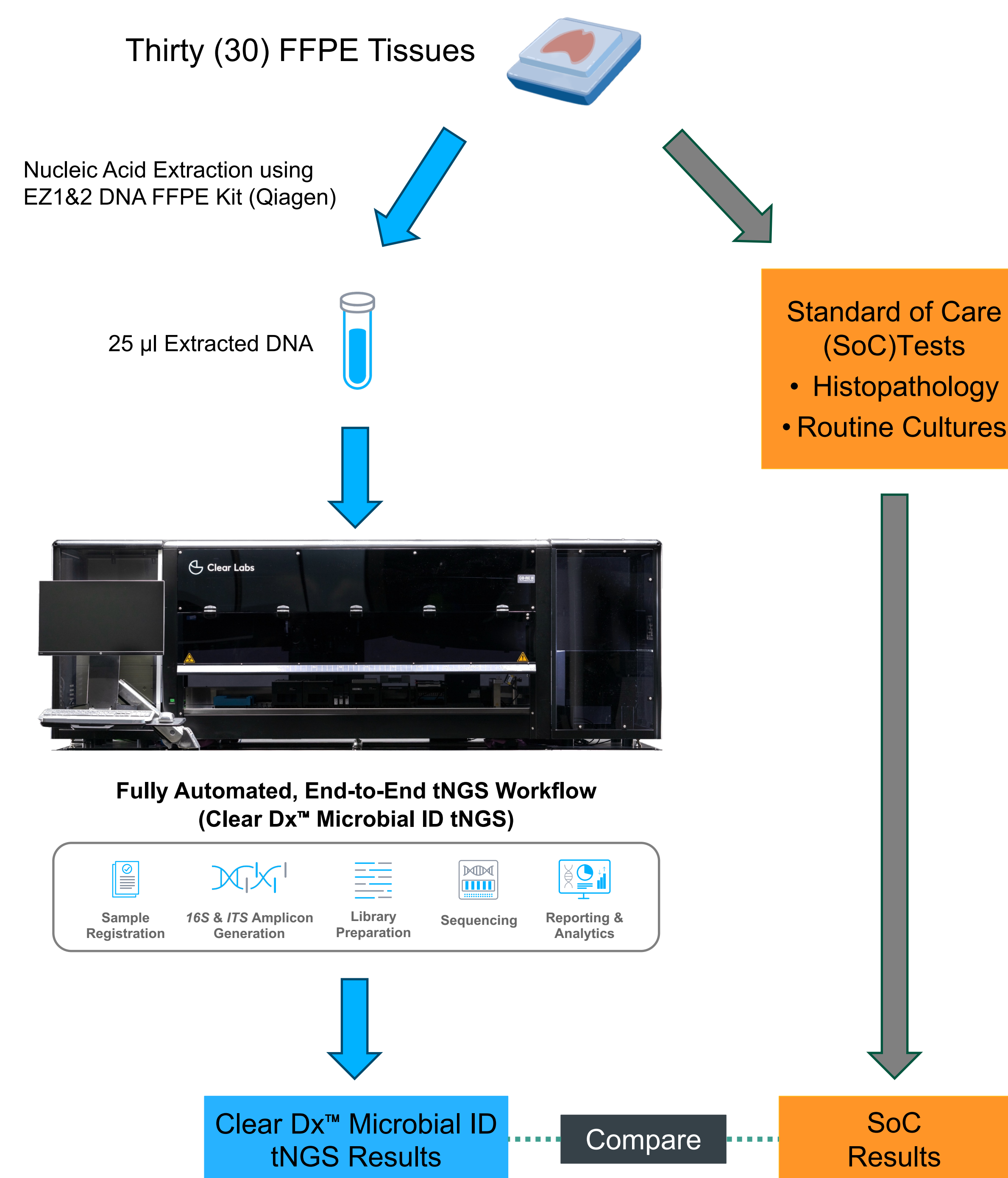
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## Introduction

Formalin-fixed, paraffin-embedded (FFPE) tissue specimens in pathology can preserve the integrity of the tissue and allow for histopathological examination to aid in the diagnosis of infectious diseases. However, exact determination of the infectious organism can be difficult. Surgical pathology may reveal morphological features suggestive of infection, but these findings alone may be inconclusive due to nonspecific staining or morphological similarities among organisms. Next generation sequencing (NGS), specifically targeted 16S rRNA and ITS gene sequencing (tNGS) has become a widely used approach for identification of various bacteria and fungi, respectively. A streamlined, automated tNGS workflow has the potential to overcome these challenges, providing higher sensitivity and broader pathogen coverage while minimizing manual steps and turnaround time. Here, we evaluated the performance of a commercially-available, fully automated 16S/ITS tNGS test on FFPE tissues of various anatomic sites.
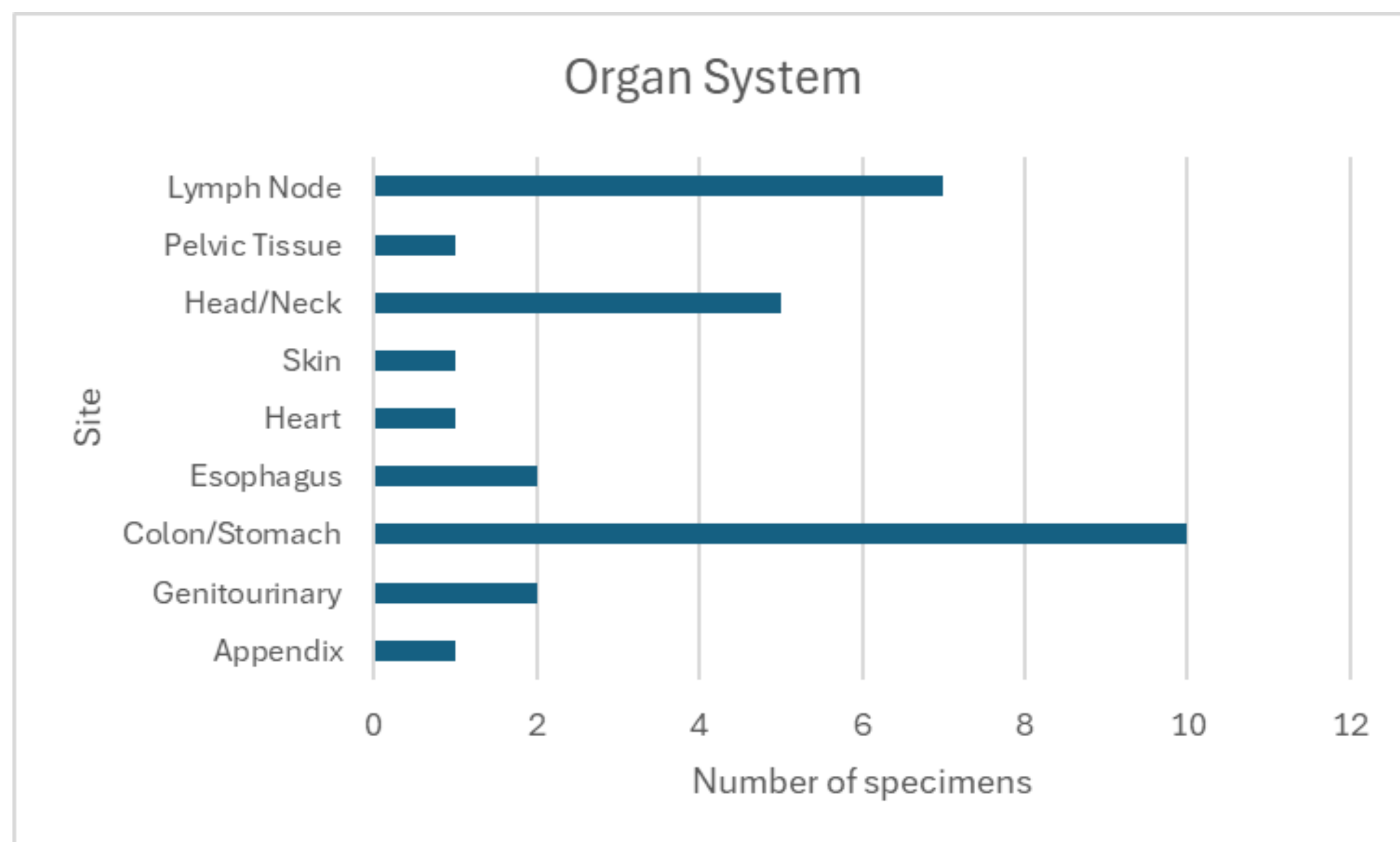
## Methods

Thirty (30) FFPE Tissues

Nucleic Acid Extraction using EZ1&2 DNA FFPE Kit (Qiagen)

25 µl Extracted DNA

Standard of Care (SoC)Tests
• Histopathology
• Routine Cultures

**Fully Automated, End-to-End tNGS Workflow (Clear Dx™ Microbial ID tNGS)**

Sample Registration | 16S & ITS Amplicon Generation | Library Preparation | Sequencing | Reporting & Analytics

Clear Dx™ Microbial ID tNGS Results → Compare → SoC Results

**Assessment:**
• Determination of concordance, sensitivity, specificity, positive and negative predictive values using SoC results as gold standard

## Results

### A: Anatomic Sources of FFPE Tested

A total of 30 specimens were tested from various anatomic sites.



Organ System — Number of specimens by Site: Lymph Node, Pelvic Tissue, Head/Neck, Skin, Heart, Esophagus, Colon/Stomach, Genitourinary, Appendix

### B: Performance of Clear Dx tNGS

| | Overall | Bacterial | Fungal |
|---|---|---|---|
| **Sensitivity** | 10/12 (83.3%) | 6/8 (75%) | 4/4 (100%) |
| **Specificity** | 18/18 (100%) | 22/22 (100%) | 26/26 (100%) |
| **Positive Predictive Value** | 10/10 (100%) | 6/6 (100%) | 4/4 (100%) |
| **Negative Predictive Value** | 18/20 (90%) | 22/24 (92%) | 26/26 (100%) |

### C: Comparison of Results Between tNGS and Pathology Review

• Clear Dx™ Microbial ID tNGS achieved an overall concordance of 93.3%.
  • 100% concordance for detection of yeast fungus.
  • 75% concordance for bacterial organisms.
  • tNGS missed two cases where SoC reported positive for acid-fast bacilli and H. pylori.

| Histopathology Observation | tNGS Concordance |
|---|---|
| **Positive (total n= 12)** | 10/12 (83.3%) |
| **Bacterial (n=8)** | 6/8 (75.0%) |
| • Positive for bacterial organisms | 2/2 (100%) |
| • Positive for intestinal spirochetosis | 4/4 (100%) |
| • Positive for mycobacterial organisms | 0/1 (0%) |
| • Positive for H. pylori | 0/1 (0%) |
| **Fungal (n=4)** | 4/4 (100%) |
| • Positive for fungal organism (e.g., yeast, spores) | 4/4 (100%) |
| **Negative (n=18)** | 18/18 (100%) |
| **Grand total (N=30)** | 28/30 (93.3%) |

| NGS Target | Read Abundance (raw) [mean, (range)] | Read Abundance (%) [mean, (range)] |
|---|---|---|
| **Overall** | 70,205 (2,141 – 206,460) | 32.38 (4.4 – 88.6) |
| **Bacteria** | | |
| • Vaginal Flora | 29,769 (13,757-73,487) | 9.57 (4.4-23.6) |
| • Pseudomonas species | 17,736 | 22.96 |
| • Brachyspira species | 81,193 (2,141-144,542) | 71.08 (42.5 – 88.6) |
| **Fungal** | | |
| • Candida albicans | 113,133 (46,218 – 206,460) | 24.12 (6.0-48.9) |
| • Nakaseomyces species | 10,367 | 14.34 |

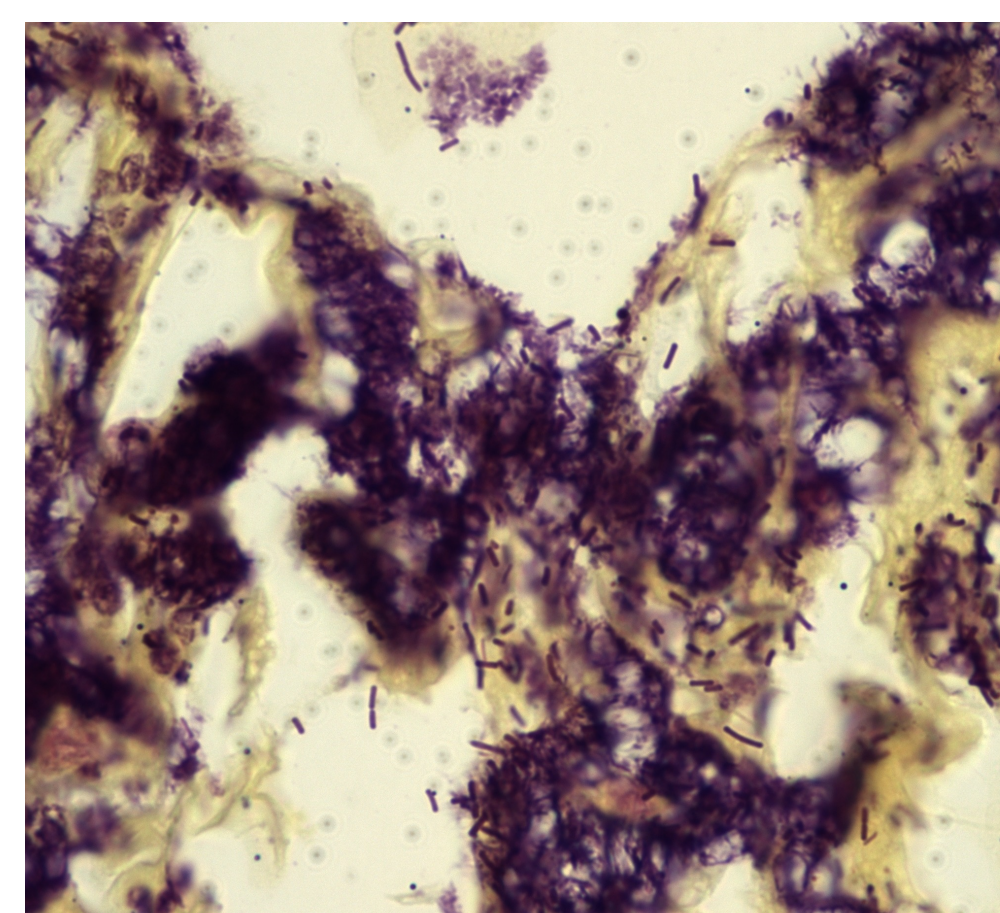### D: Examples of Histopathological Findings and Respective tNGS Results
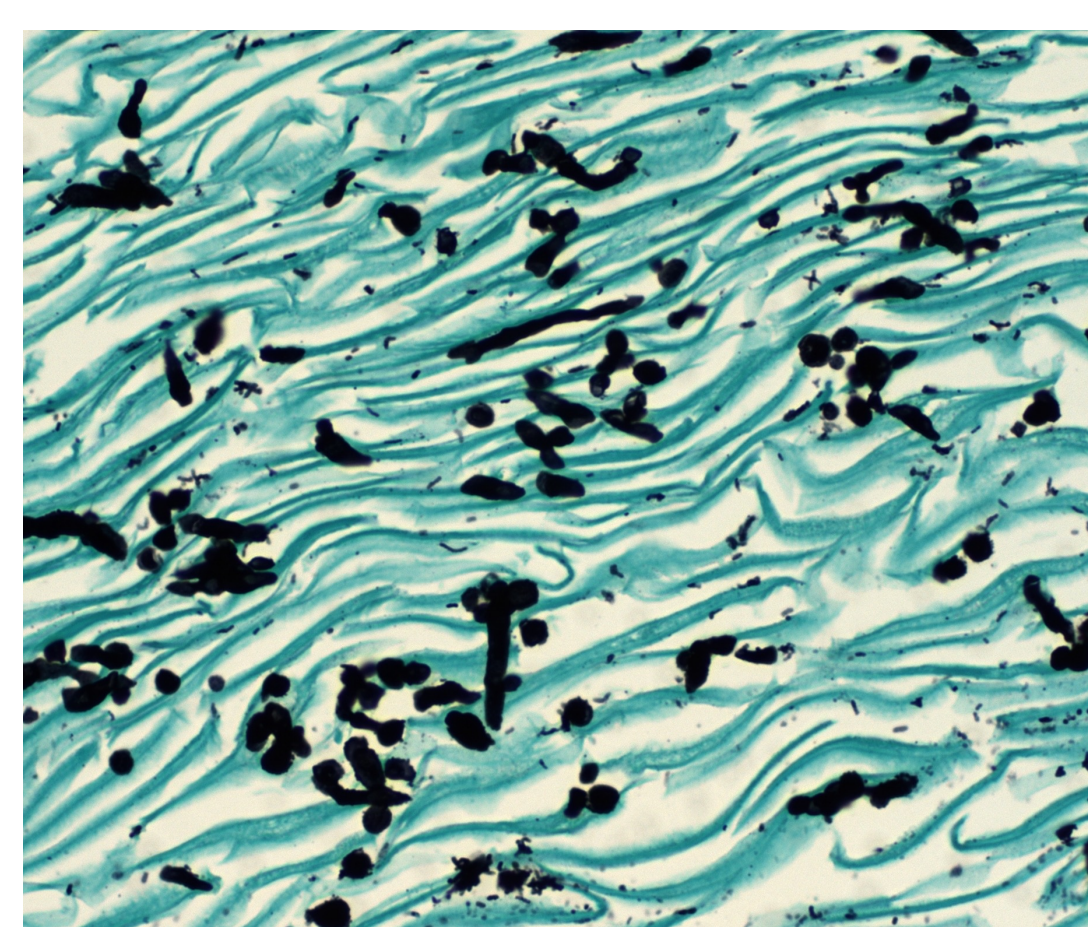


**Stomach Biopsy**
• Path report: Positive for fungal organism consistent with Candida
• tNGS: Candida albicans (reads: 46218; 6%)
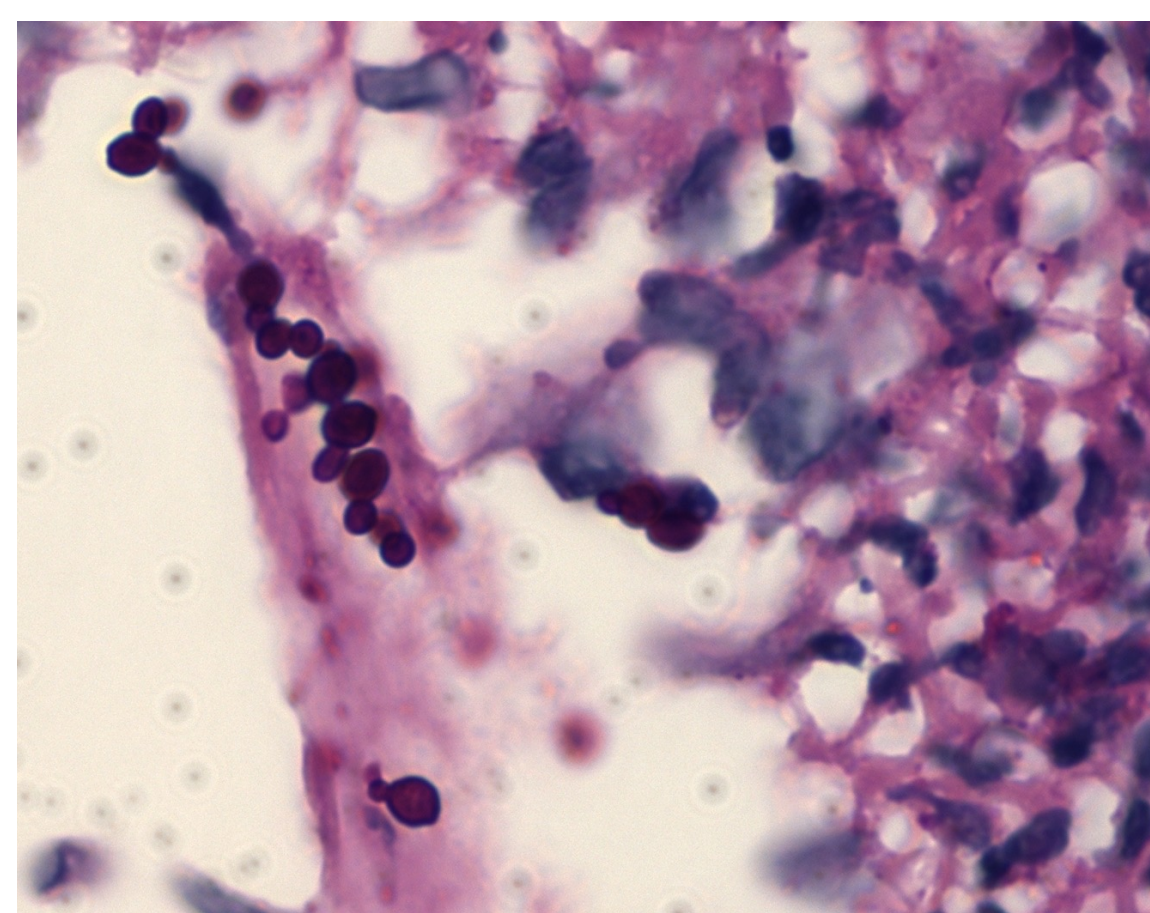
GMS stain



**Stomach Biopsy**
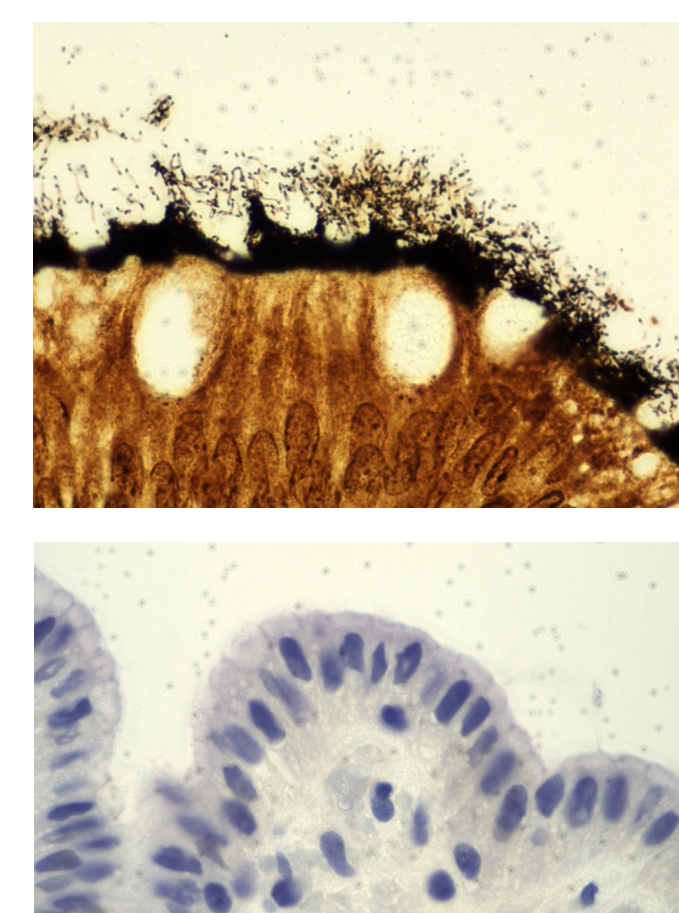• Path report: Positive for fungal spores
• tNGS: Nakaseomyces spp. (reads: 10,367; 4%)

PAS stain



**Cervix Biopsy**
• Path report: Degenerative squamous epithelium with bacteria
• tNGS: Vaginal flora [Lactobacillus spp.. (reads: 73,487; 24%); Achromobacter spp. (reads: 15,573; 5%); Cutibacterium spp. (reads: 13,757; 4%)]

Gram stain



**Stomach Biopsy**
• Path report: Positive for intestinal spirochetosis, negative for H. pylori
• tNGS: Brachyspira aalborgi (reads: 96,879; 82%)

Warthin-Starry

## Conclusions

• The fully automated Clear Dx™ Microbial ID tNGS assay is a promising approach for accurate and unbiased fungal and bacterial identification from FFPE
• Performance of Clear Dx™ Microbial ID tNGS assay is comparable from various anatomical sites.
• Ongoing runs with additional FFPE specimens will increase the power of this study.
• Further evaluation of extraction protocols and inclusion of specimens with more diverse pathological findings (e.g., fungal hyphae) will provide insights into the accuracy.
• Sequencing approaches with appropriate diagnostic stewardship can complement SOC practices and will have a significant impact in infectious disease management decisions.

References:
• Larkin et. al., Amplicon-Based Next-Generation Sequencing for Detection of Fungi in Formalin-Fixed, Paraffin-Embedded Tissues: Correlation with Histopathology and Clinical Applications. J Mol Diagn. 2020 Oct;22(10):1287-1293. doi: 10.1016/j.jmoldx.2020.06.017. Epub 2020 Jul 30. PMID: 32738297.
• Lao H-Y, et al. The clinical utility of Nanopore 16S rRNA gene sequencing for direct bacterial identification in normally sterile body fluids. Frontiers in Microbiology. 2024;14:1324494. DOI: 10.3389/fmicb.2023.1324494.
• Flurin L, et al. Targeted Metagenomic Sequencing-based Approach Applied to 2146 Tissue and Body Fluid Samples in Routine Clinical Practice. Clin Infect Dis. 2022 Nov 14;75(10):1800-1808. doi: 10.1093/cid/ciac247. PMID: 35362534; PMCID: PMC9662179.