

## ABSTRACT

**INTRODUCTION:** Currently there are no standard incubation times stated in the Clinical Microbiology Procedures Handbook or the Manual of Clinical Microbiology. ASM's Cumitechs do provide some guidance indicating that plate readings are performed once daily. And there are no recommendations for incubation times when using Smart Incubators where specimens are continuously incubated without the need to take plates from the incubator and disrupt the atmospheric conditions as with manual incubation. There are several systems commercially available that offer continuous incubation, including the Copan WASPLab. This study was undertaken to evaluate when cultures were ready for workup when incubated in Copan Smart Incubators as a part of the WASPLab.

**METHODS:** A total of 374 residual patient specimens were included in the study and included sterile sources (106-body fluids and tissues), abscesses (49), deep wounds (43), superficial wounds (44), respiratory (28), and urines (104). Specimens were cultured and read manually at standard incubation times (18-24hrs, 48hrs, & 72hrs) and setup and incubated in the WASPLab. Culture images were taken in the WASPLab at 10hrs, 12hrs, 14hrs, 16hrs, 18hrs, 20hrs, 22hrs, 24hrs, 36hrs, and 72hrs. Culture images from WASPLab were read by technologists who determined when the cultures were first ready to be worked up. **RESULTS:** Considering all non-urine specimens, between 52-60% of cultures were ready to be worked up at 16hrs and between 92-100% were ready to be worked up after 24hrs of incubation on WASPLab. With urine specimens 6% were ready to be worked up after 12hrs, 81% after 14hrs, and 90% after 16hrs of incubation on WASPLab; 5 specimens were ready to be worked up at 18hrs but were present at 16hrs and could be sub-cultured for work up the following day. No additional significant growth occurred in any culture type after 36hrs of incubation.

**DISCUSSION:** Based on the data from this study, reading times for non-urine specimen were selected at 16hrs for an initial read, 24hrs for an intermediate read, and 36hrs for a final reading time. For urine specimen 16hrs was selected for a single and final read. Limitations to the study included: 1) very few fastidious organisms were included in the study. For example, respiratory specimens available at the study time did not contain pathogens such as *S. pneumoniae*, *H. influenzae* or *S. maltophilia*. Further studies are needed to include these more fastidious organisms. In addition, performance of susceptibility testing and MALDI-TOF identifications need to be assessed at these earlier timepoints. **SUMMARY:** The WASPLab continuous incubation system allows for standardized and earlier plate reading times for all specimen types than what is currently used with manual incubation. Decreasing incubation times will speed time to final culture results including identification and susceptibility testing with additional verification testing.

## INTRODUCTION

Currently there are no standard incubation times stated in the Clinical Microbiology Procedures Handbook or the Manual of Clinical Microbiology. ASM's Cumitechs do provide some guidance indicating that plate readings are performed once daily. And there are no recommendations for incubation times when using Smart Incubators where specimens are continuously incubated without the need to take plates from the incubator and disrupt the atmospheric conditions as with manual incubation. It is widely accepted that cultures grown in these constant conditions grow faster and can be workup sooner even though there is no published data that cultures grown on routine media can be worked up earlier. This study was undertaken to evaluate when cultures were ready for workup when incubated in Copan Smart Incubators as a part of the WASPLab.

## METHODS

- Patient specimens were cultured and read manually at standard incubation times (18-24hrs, 48hrs, 72hrs) during WASPLab validation studies between October and December of 2023.
- Residual patient specimens included 106 sterile sources (body fluids, joint fluids, and tissues), 49 abscesses, 43 deep wounds, 44 superficial wounds, 28 respiratory sources and 104 urines.
- All specimens were plated and incubated using manual methods as well as being plated by WASP and incubated in WASPLab.
- The WASPLab took digital culture images at the following time points of incubation: 10hrs, 12hrs, 14hrs, 16hrs, 18hrs, 20hrs, 22hrs, 24hrs, 36hrs, and 72hrs.
- Digital culture images from the WASPLab were read by technologists who determined when the culture was ready to have work up started.
- Results interpreted from digital images from the WASPLab were compared to results of the standard manual culture and reading methods for identification of organisms.

## RESULTS

### Sterile sources – Chart 1 / Figure 1

There were 25 positive cultures in this group; 15 of 25 positive cultures (60%) were ready to read at 16hrs and 23 of 25 positive cultures (92%) were ready to read at 24hrs. The 2 cultures ready to read at 36hrs had suspected colonies of aerotolerant *Actinomyces* spp breakthrough and were considered non-significant. No additional significant organisms were seen upon incubation past 36hrs. The WASPLab image times chosen were 16hrs, 24hrs, and 36hrs for this source.

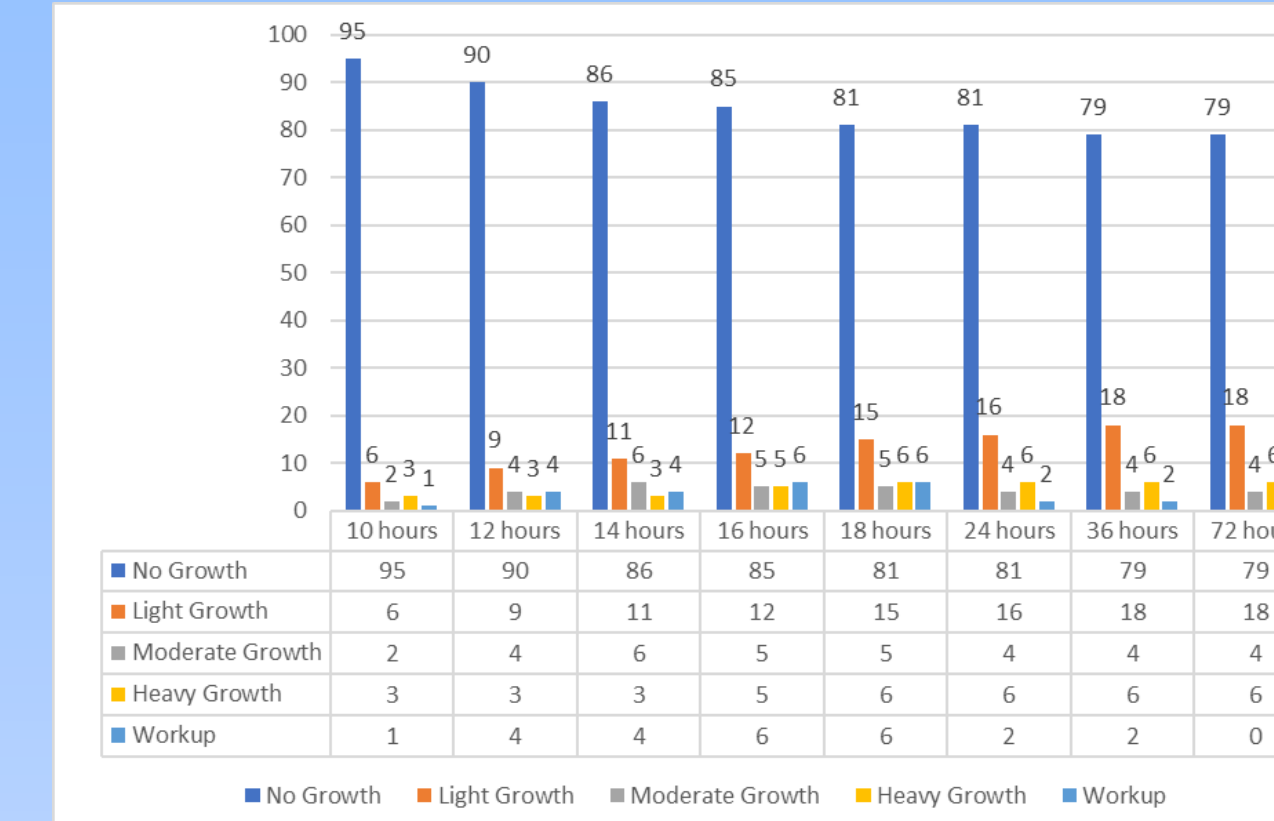


Chart 1: Sterile Sources

### Abscesses – Chart 2 / Figure 2

There were 28 positive cultures in this group; 15 of 28 positive cultures (54%) were ready to read at 16hrs, and 26 of 28 positive cultures (93%) were ready to read at 24hrs. Two single colonies appeared at 36hrs on the remaining 2 cultures (1 colony *E. coli*; 1 colony coagulase-negative staphylococci species). No additional significant organisms were seen upon incubation past 36hrs. The WASPLab image times chosen were 16hrs, 24hrs, and 36hrs for this source.

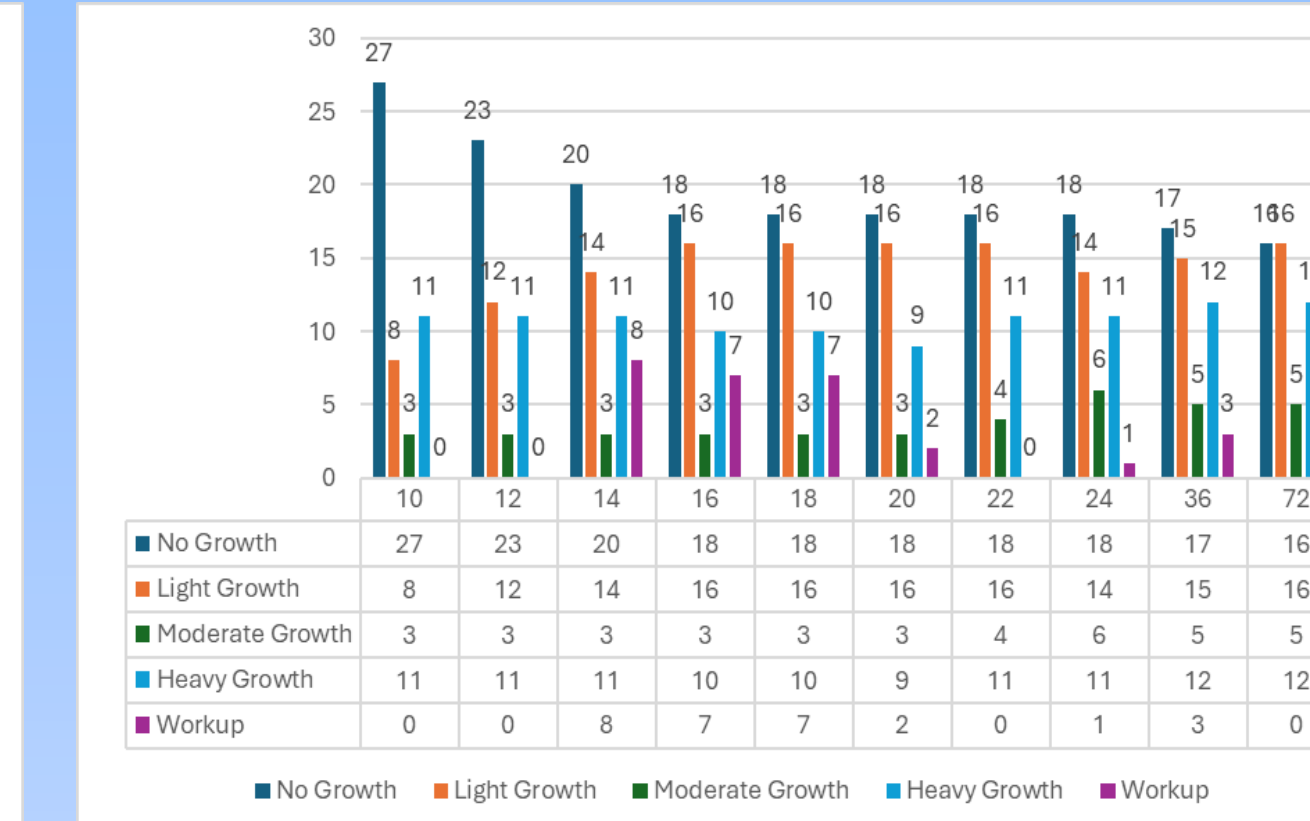


Chart 2: Abscesses

### Deep Wounds – Chart 3

There were 25 positive cultures in this group; 13 of 25 positive cultures (52%) were ready to read at 16hrs, and 25 of 25 positive cultures (100%) were ready to read at 24hrs. No additional significant organisms were seen upon incubation past 36hrs. The WASPLab image times chosen were 16hrs, 24hrs, and 36hrs for this source.

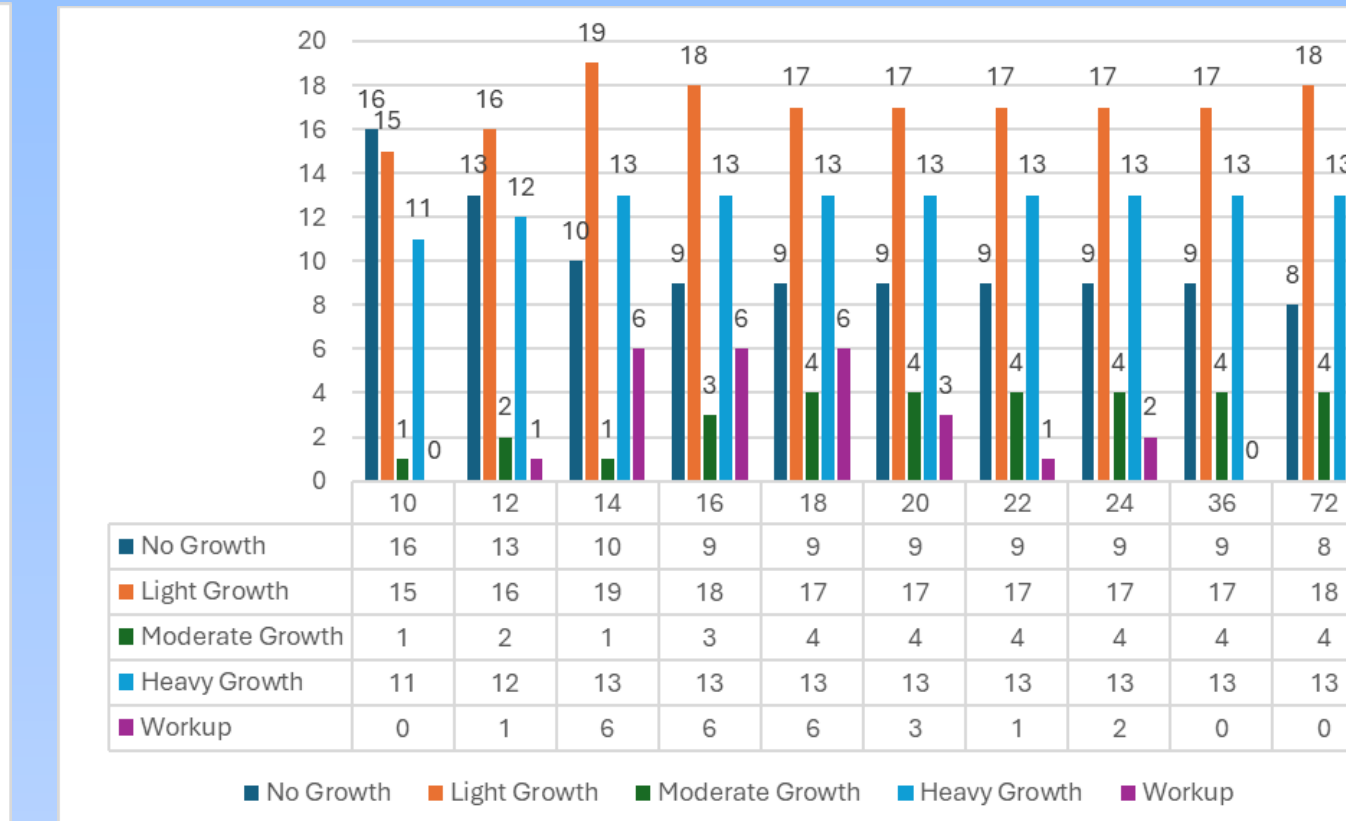


Chart 3: Deep Wounds

### Superficial Wounds – Chart 4

There were 25 positive cultures in this group; 13 of 22 positive cultures (59%) were ready to read at 16hrs, and 25 of 25 (100%) were ready to read at 24hrs. No additional significant organisms were seen upon incubation past 36hrs. The WASPLab image times chosen were 16hrs, 24hrs, and 36hrs for this source.

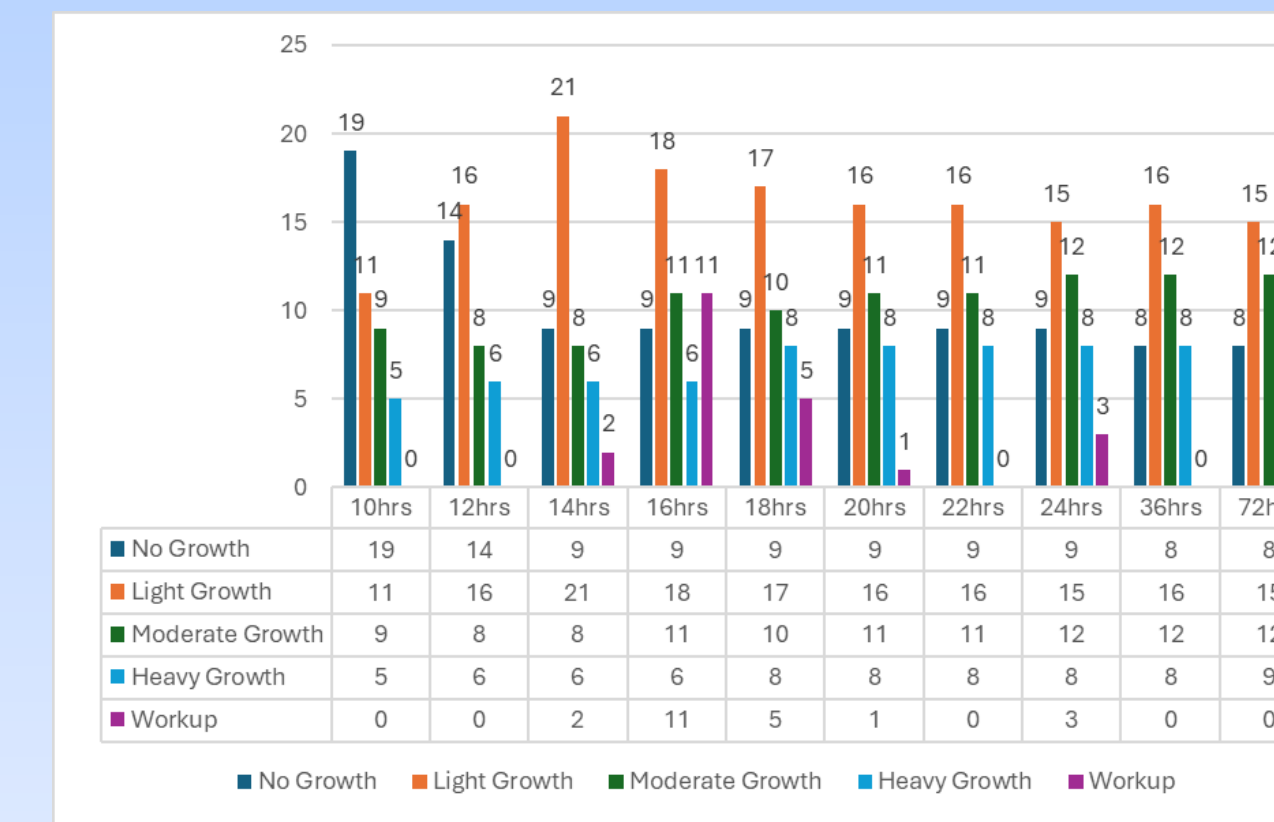


Chart 4: Superficial Wounds

### Respiratory – Chart 5

There were 9 positive cultures in this group; 7 of 9 positive cultures (59%) were ready to read at 14hrs, and 9 of 9 (100%) were ready to read at 16hrs. The WASPLab image times chosen were 16hrs, 24hrs, and 36hrs for this source.

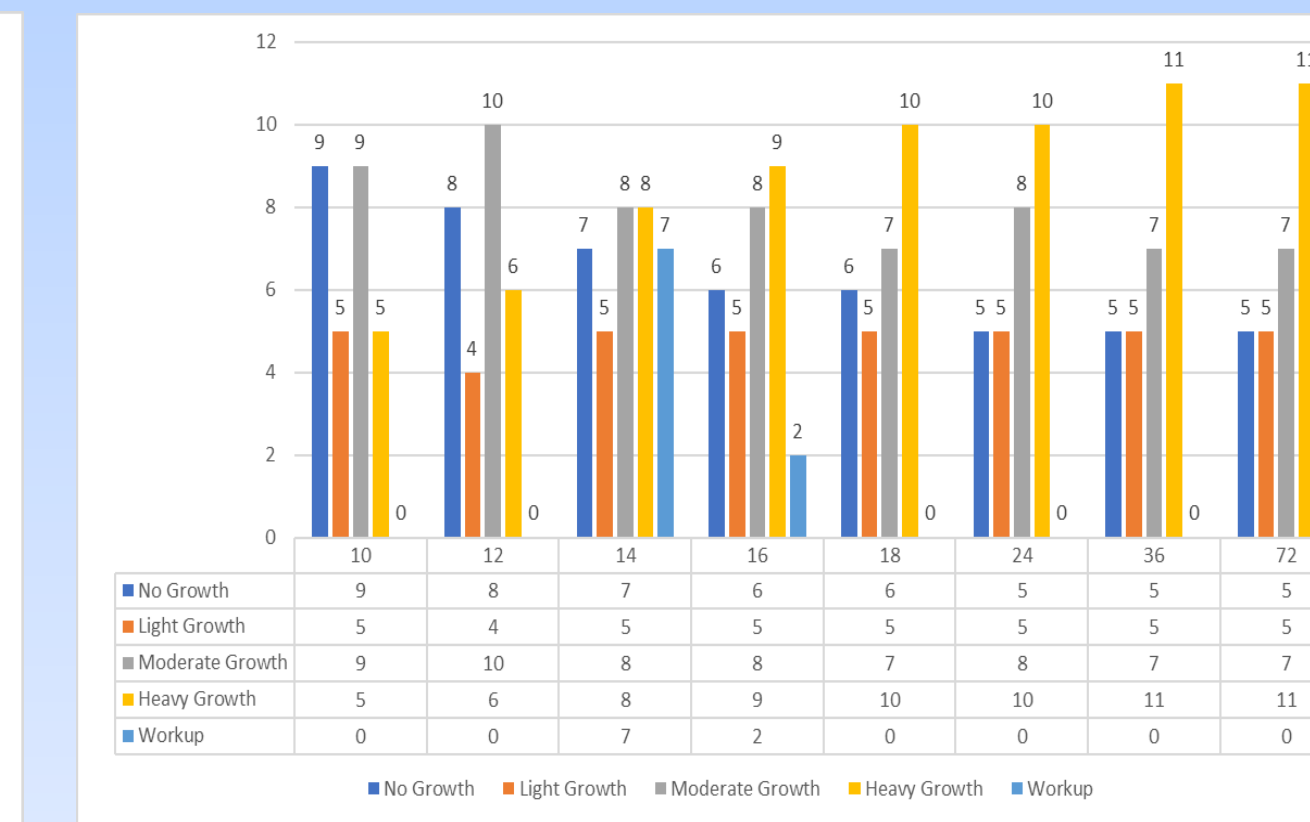


Chart 5: Respiratory

### Urine – Chart 6

There were 53 positive cultures in this group; 3 of 53 positive cultures (6%) were ready to read at 12hrs, 43 of 53 (81%) were ready to read at 14hrs, 48 of 53 (90%) were ready to read at 16hrs, and 5 were ready at 18hrs but were also present at 16hrs and were ready to be sub-cultured for workup the following next day. The WASPLab image time chosen were 16hrs for this source.

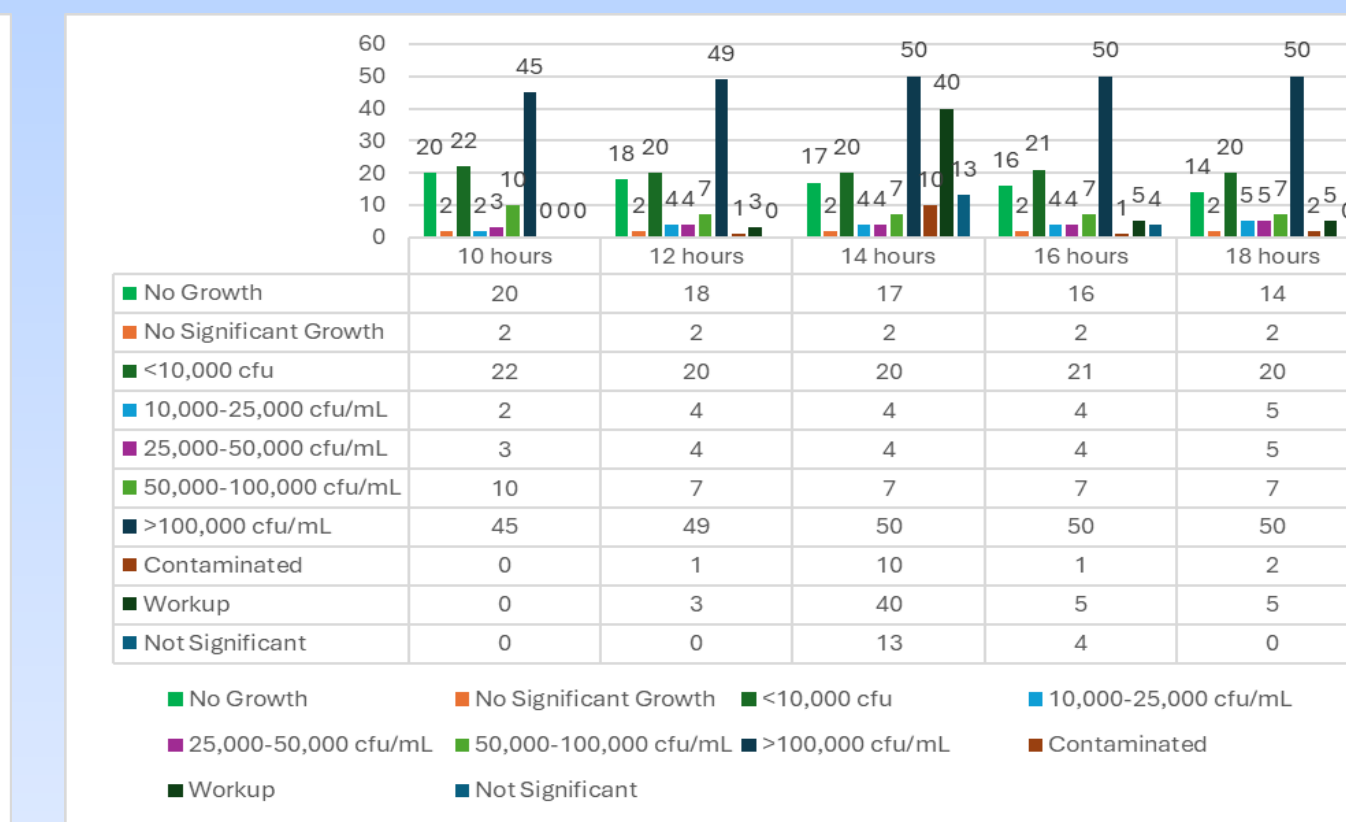


Chart 6: Urine

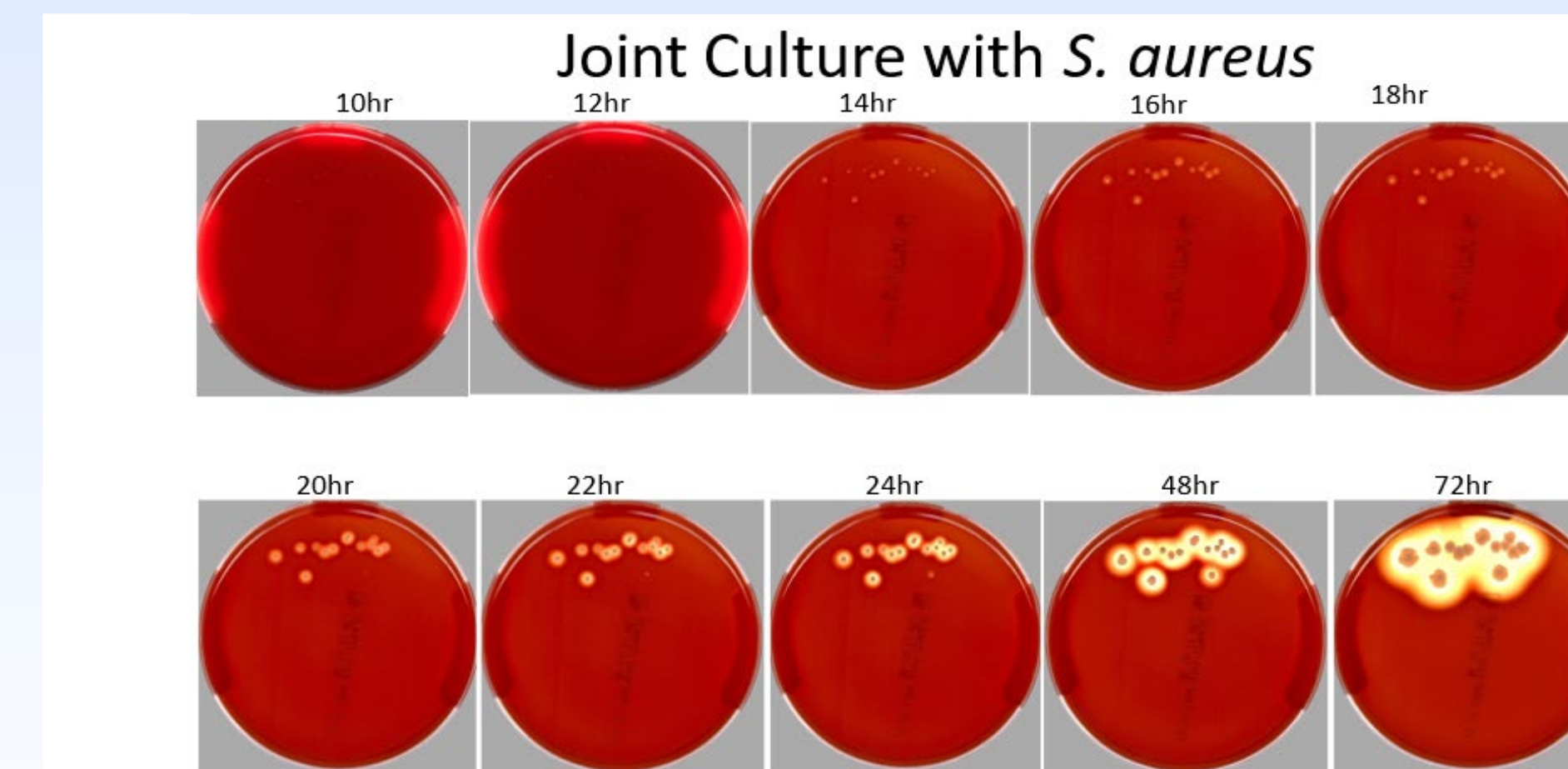


Figure 1

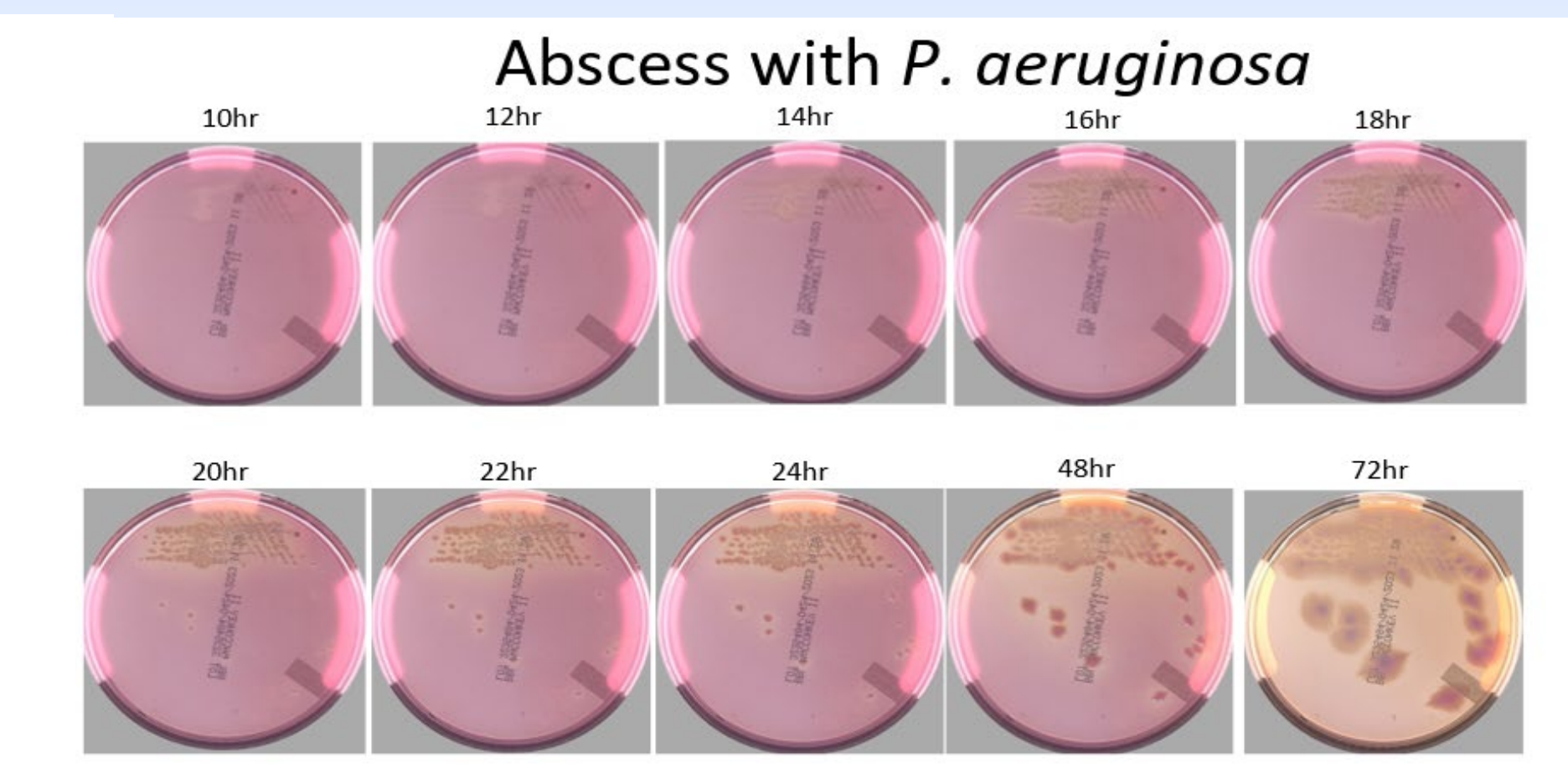


Figure 2

## CONCLUSIONS

- This study showed that for all specimen types cultured in the laboratory, 50% or more of the cultures could be read after 16hrs of incubation in the WASPLab with no significant changes in quantitation of the cultures occurring after 16hrs. No additional growth in any cultures occurred after 36hrs of incubation in the WASPLab. This data shows that cultures from non-urine sources can be first read at 16hrs and finalized at 36hrs; urine cultures can be finalized with one reading at 16hrs.
- Limitations: Very few fastidious organisms were included in the specimens sampled during the study timeframe. In addition, performance of susceptibility testing and MALDI-TOF identifications need to be assessed at these earlier reading times for accuracy.