**A high-throughput microbial growth data assembly and analysis protocol to elucidate the strain variability of *Listeria monocytogenes* in NaCl stress**

Mariella Aalto-Araneda, Anna Pöntinen, Maiju Pesonen, Jukka Corander, Annukka Markkula, Taurai Tasara, Roger Stephan, Hannu Korkeala

Supplemental figures

**Content:**
S1. Variation between growth curves of the same strains in pilot measurements performed at NaCl concentrations of 6.5%, 7.5% and 8.5%, respectively.

S2. Selection of strain growth curves to illustrate the technical oscillation due to the measurement equipment appearing as sharp peaks in some absorbance measurements taken at 15-minute intervals.

S3. *L. monocytogenes* growth in hours: replicates of control strains from each run color-coded by inner and outermost honeycomb plate wells.

S4. *L. monocytogenes* growth in hours: replicates of control strains from each run color-coded by laboratory technicians.

S5. *L. monocytogenes* growth in hours: replicates of control strains from inner wells color-coded by laboratory technician.

S6. *L. monocytogenes* growth in hours: replicates of control strains from inner wells color-coded by laboratory technician, including mean.

S7. Difference between laboratory technicians by hour for both salt control curves and their mean.

S8. *L. monocytogenes* growth in hours, not corrected for batch effect.

S9. *L. monocytogenes* growth in hours, corrected for batch effect.

S11. Honeycomb plate loading chart for Bioscreen growth experiments.

S12. Distribution of *Listeria monocytogenes* strains (n = 388) into growth clusters determined by $OD_{600}$ as clustering variable (alternative clustering outcome 1).

S13. Distribution of *Listeria monocytogenes* strains (n = 388) into growth clusters determined by lag phase (λ), growth rate (μ), maximum growth density (MaxOD), and area under the curve (AUC) as clustering variables (alternative clustering outcome 2).

S14. Distribution of *Listeria monocytogenes* strains (n = 388) into growth clusters determined by lag phase (λ), growth rate (μ), and maximum growth density (MaxOD) as clustering variables (alternative clustering outcome 3).

S15. Distribution of *Listeria monocytogenes* strains (n = 388) into growth clusters determined by area under the curve (AUC) as clustering variable (alternative clustering outcome 4).
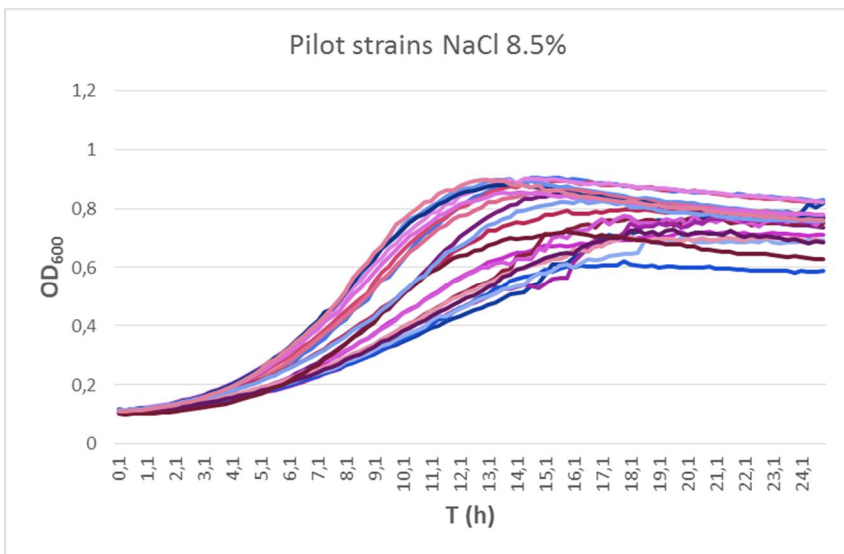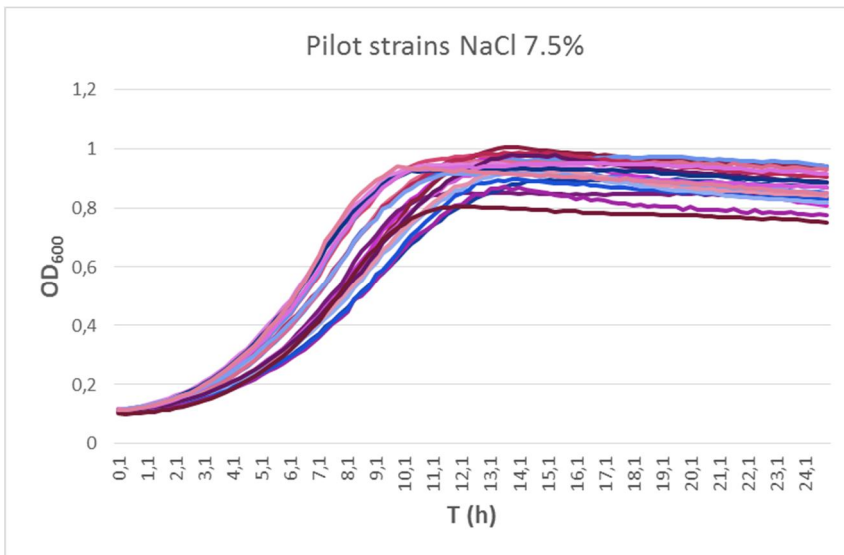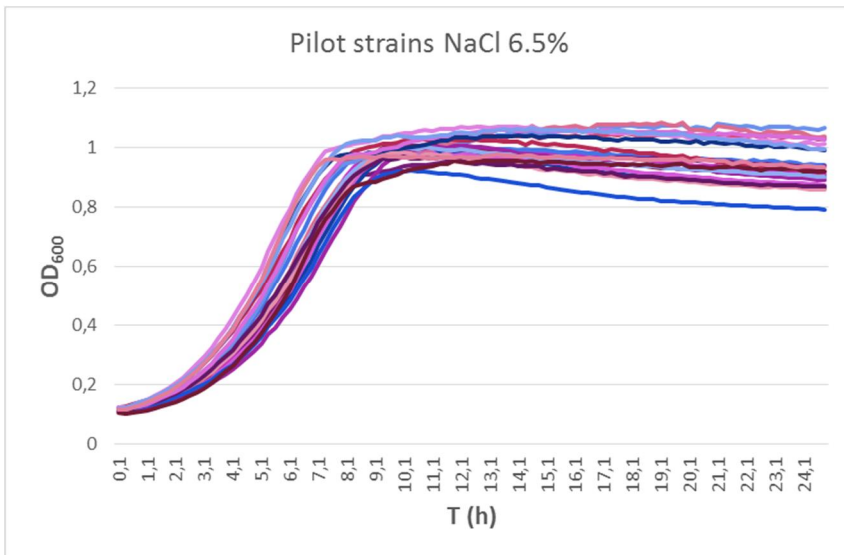
Supplemental Figure S1. Variation between growth curves of the same strains in pilot measurements performed at NaCl concentrations of 6.5%, 7.5% and 8.5%, respectively.
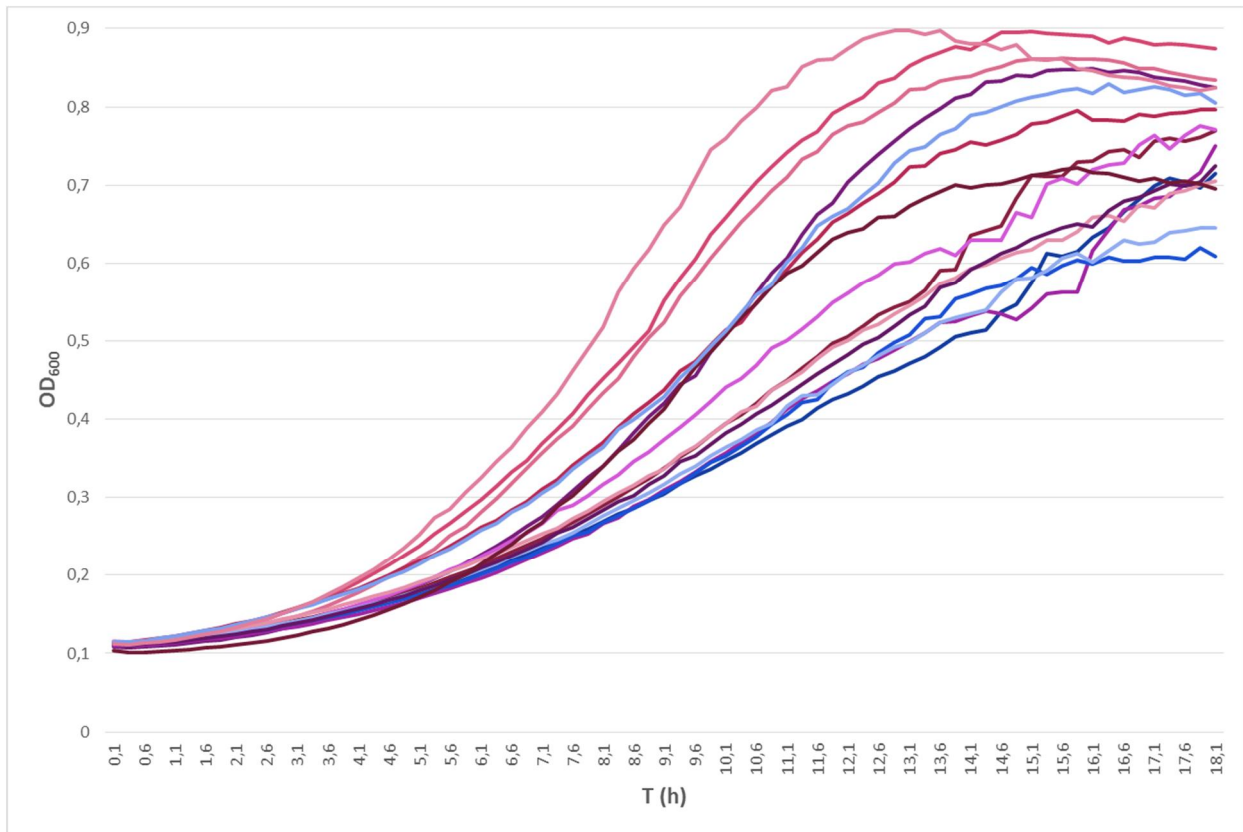
Supplemental Figure S2. Selection of strain growth curves to illustrate the technical oscillation due to the measurement equipment appearing as sharp peaks in some absorbance measurements taken at 15-minute intervals.
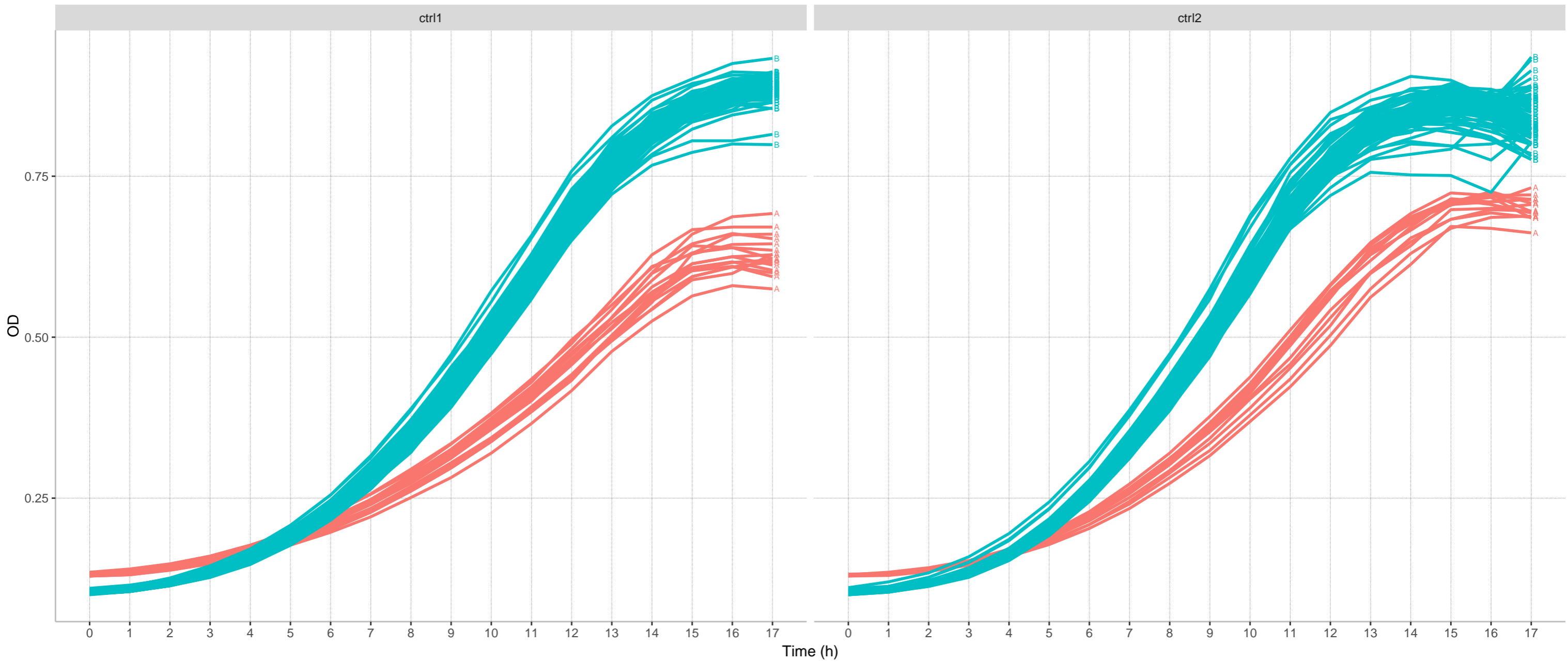
S3. NaCl 9.0%, L. monocytogenes growth in hours, replicates of control strains from each run, color–coded by inner (red) and outer (blue) honeycomb plate wells
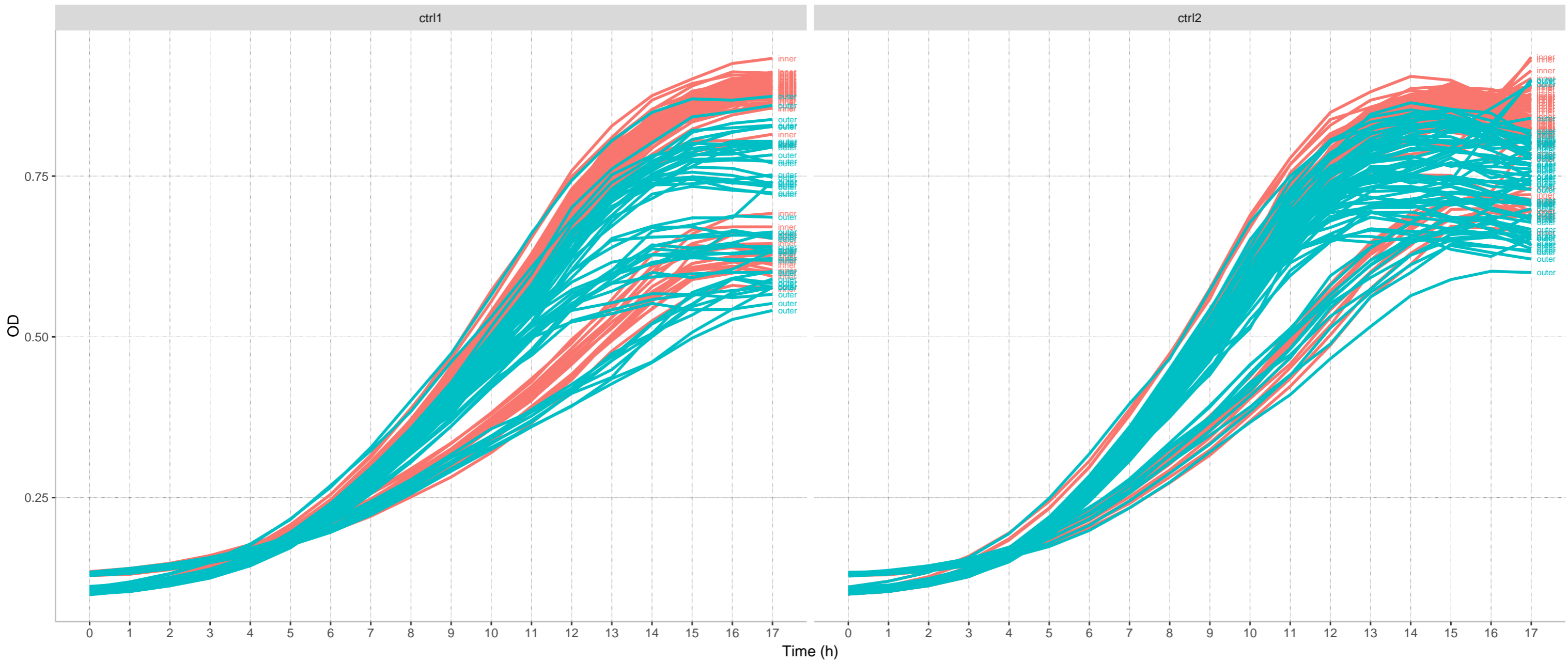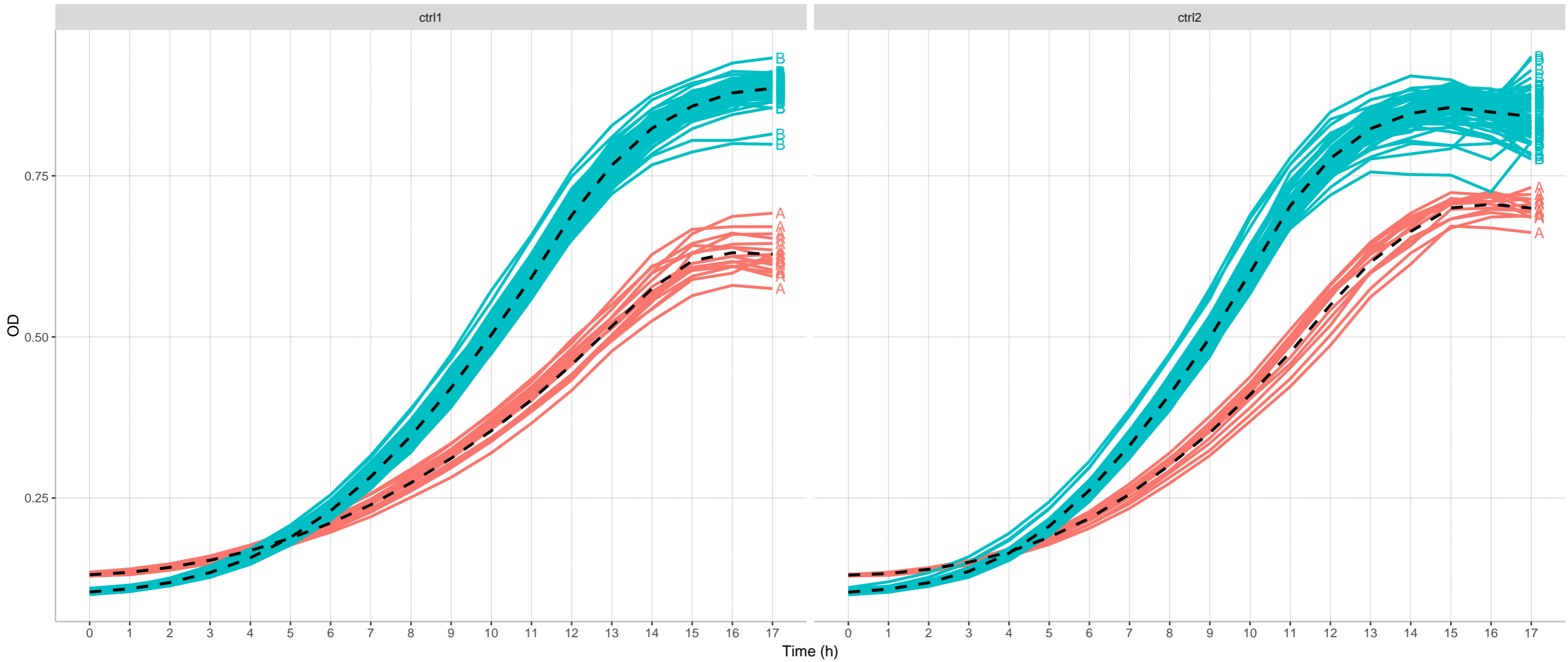
S4. NaCl 9.0%, L. monocytogenes growth in hours, replicates of control strains from each run, color−coded by laboratory technician: A (red), B (blue)
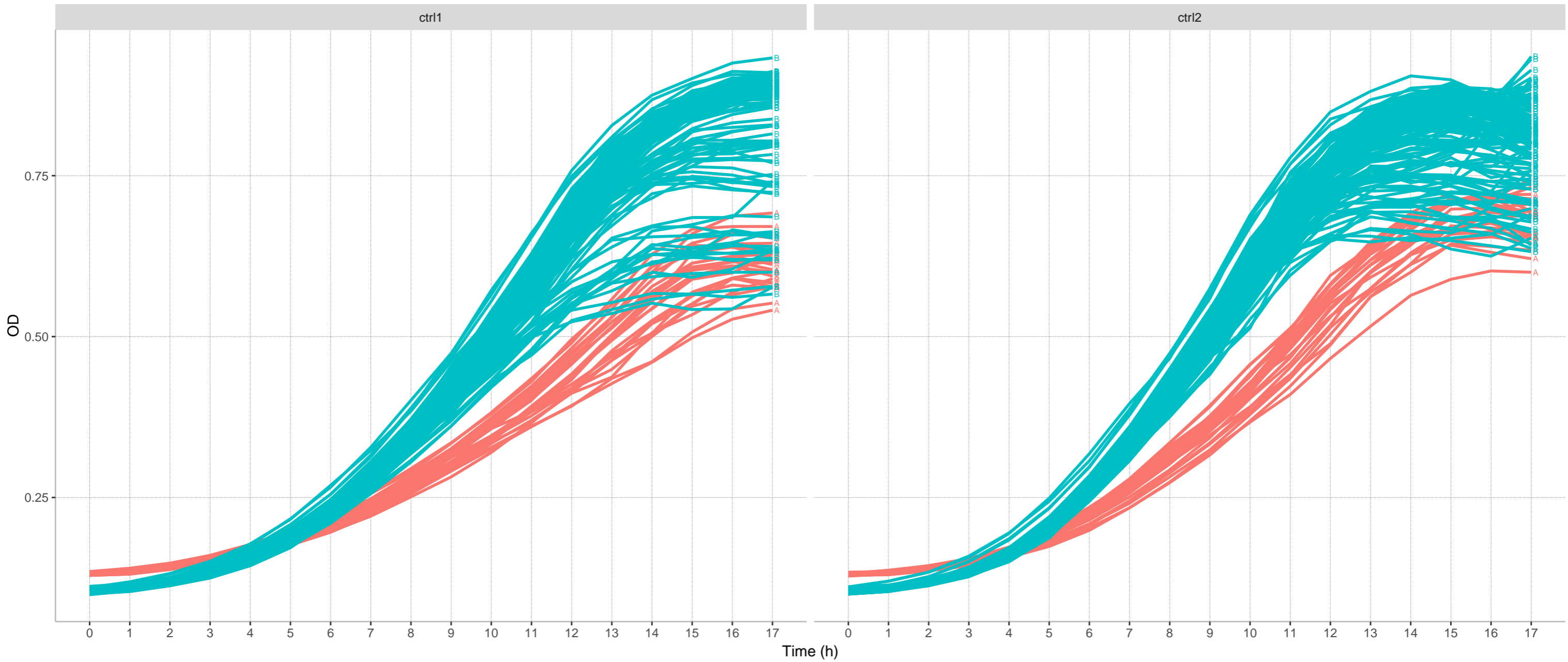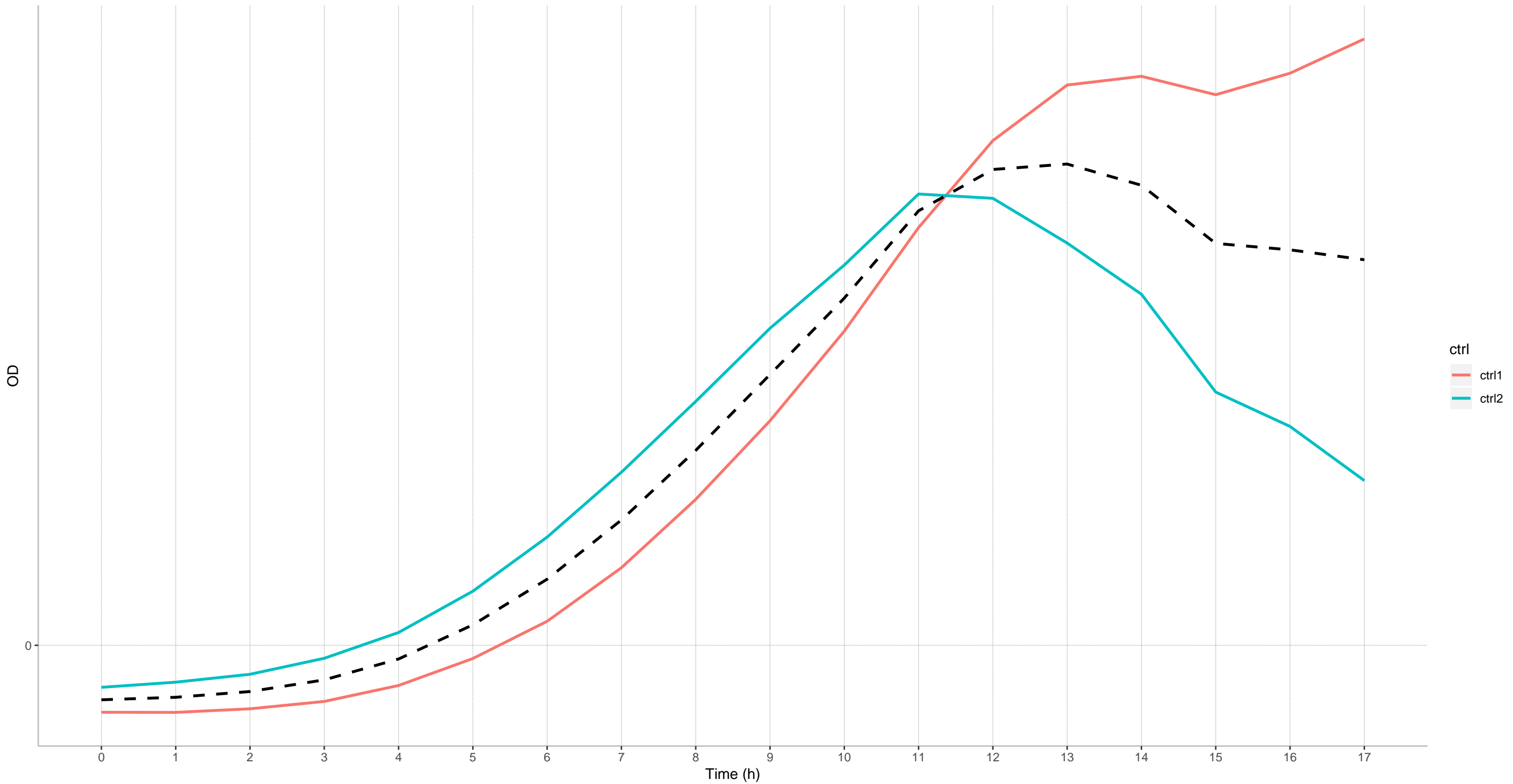
S5. NaCl 9.0%, L. monocytogenes growth in hours, replicates of control strains from inner wells only, color−coded by laboratory technician: A (red), B (blue)
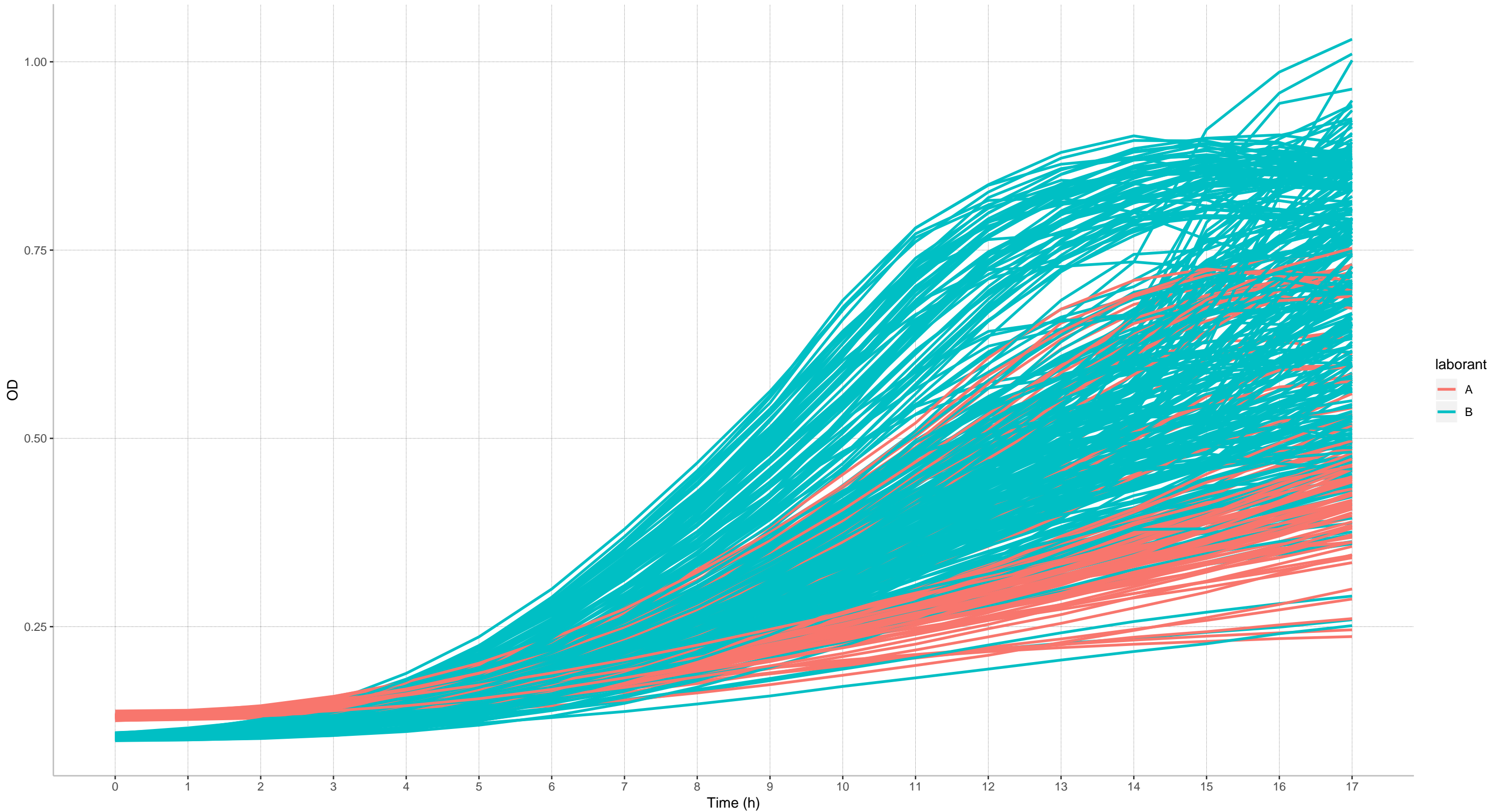
S6. NaCl 9.0%, L. monocytogenes growth in hours, replicates of control strains from inner wells only, color−coded by laboratory technician A (red) & B (blue), dashed line represents mean
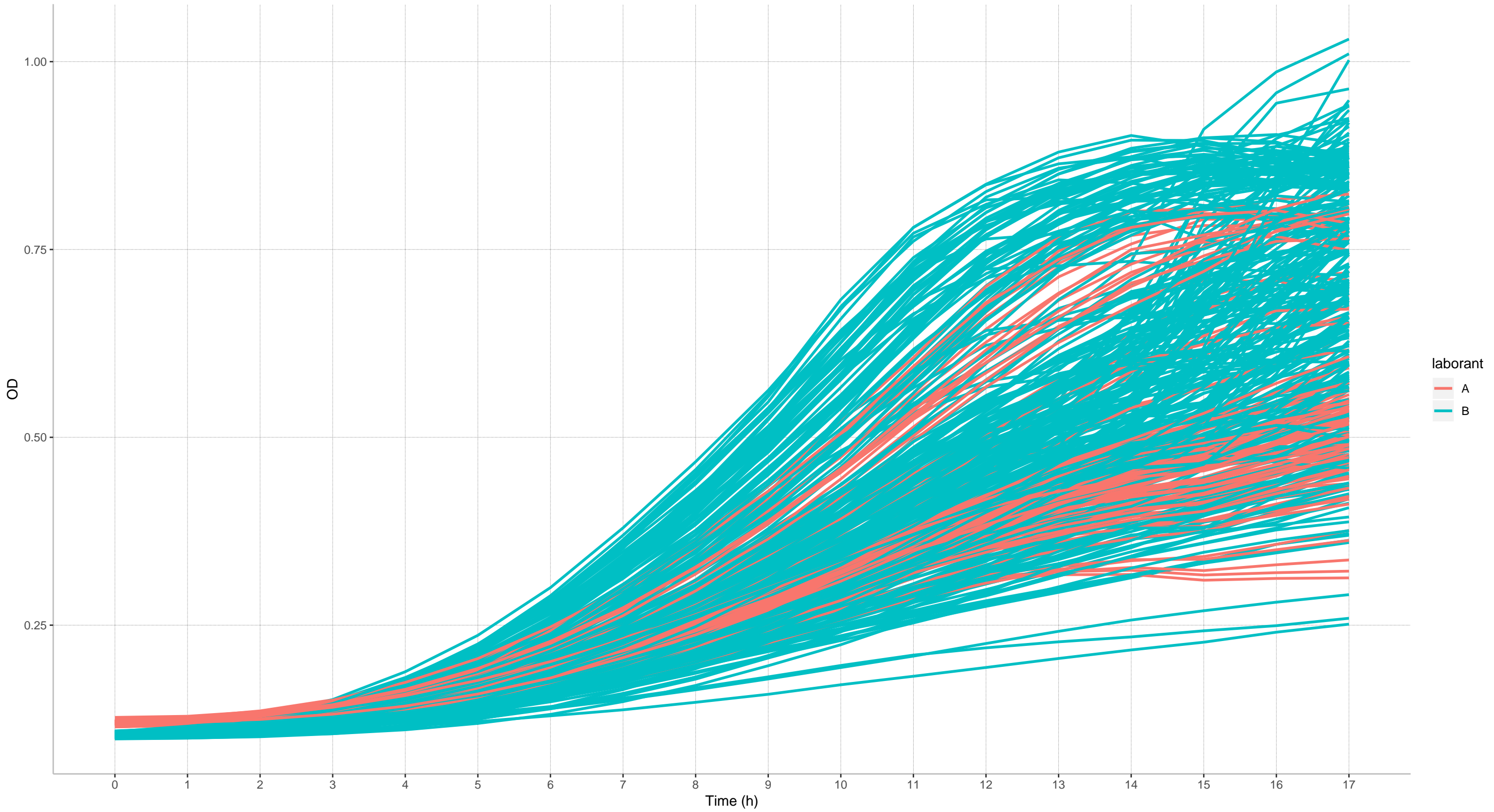
S7. Difference between lab technicians (A−B) by hour, for both salt control curves and their mean (dashed line)

S8. NaCl 9.0%, L. monocytogenes growth in hours, not corrected for bacth effect, 388 strains color–coded by lab technician (A–B)

S9.    NaCl 9.0%, L. monocytogenes growth in hours, corrected for batch effect, 388 strains color−coded by lab technician (A−B)

|   | 101 | 111 | 121 | 131 | 141 | 151 | 161 | 171 | 181 | 191 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) |
| 2 | Blank (BHI with 9% NaCl) | control 1 B1T1 | control 1 B1T2 | control 2 B1T1 | control 2 B1T2 | S1B1T1 | S1B1T2 | S2B1T1 | S2B1T2 | Blank (BHI with 9% NaCl) |
| 3 | Blank (BHI with 9% NaCl) | S3B1T1 | S3B1T2 | S4B1T1 | S4B1T2 | S5B1T1 | S5B1T2 | S6B1T1 | S6B1T2 | Blank (BHI with 9% NaCl) |
| 4 | Blank (BHI with 9% NaCl) | S7B1T1 | S7B1T2 | S8B1T1 | S8B1T2 | control 1 B2T1 | control 1 B2T2 | control 2 B2T1 | control 2 B2T2 | Blank (BHI with 9% NaCl) |
| 5 | Blank (BHI with 9% NaCl) | S1B2T1 | S1B2T2 | S2B2T1 | S2B2T2 | S3B2T1 | S3B2T2 | S4B2T1 | S4B2T2 | Blank (BHI with 9% NaCl) |
| 6 | Blank (BHI with 9% NaCl) | S5B2T1 | S5B2T2 | S6B2T1 | S6B2T2 | S7B2T1 | S7B2T2 | S8B2T1 | S8B2T2 | Blank (BHI with 9% NaCl) |
| 7 | Blank (BHI with 9% NaCl) | control 1 B3T1 | control 1 B3T2 | control 2 B3T1 | control 2 B3T2 | S1B3T1 | S1B3T2 | S2B3T1 | S2B3T2 | Blank (BHI with 9% NaCl) |
| 8 | Blank (BHI with 9% NaCl) | S3B3T1 | S3B3T2 | S4B3T1 | S4B3T2 | S5B3T1 | S5B3T2 | S6B3T1 | S6B3T2 | Blank (BHI with 9% NaCl) |
| 9 | Blank (BHI with 9% NaCl) | S7B3T1 | S7B3T2 | S8B3T1 | S8B3T2 | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) |
| 10 | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) | Blank (BHI with 9% NaCl) |

Supplemental Figure S11. Honeycomb plate loading chart for Bioscreen growth experiments. S = strain number; B = biological replicate 1-3; T = technical replicate 1-2.
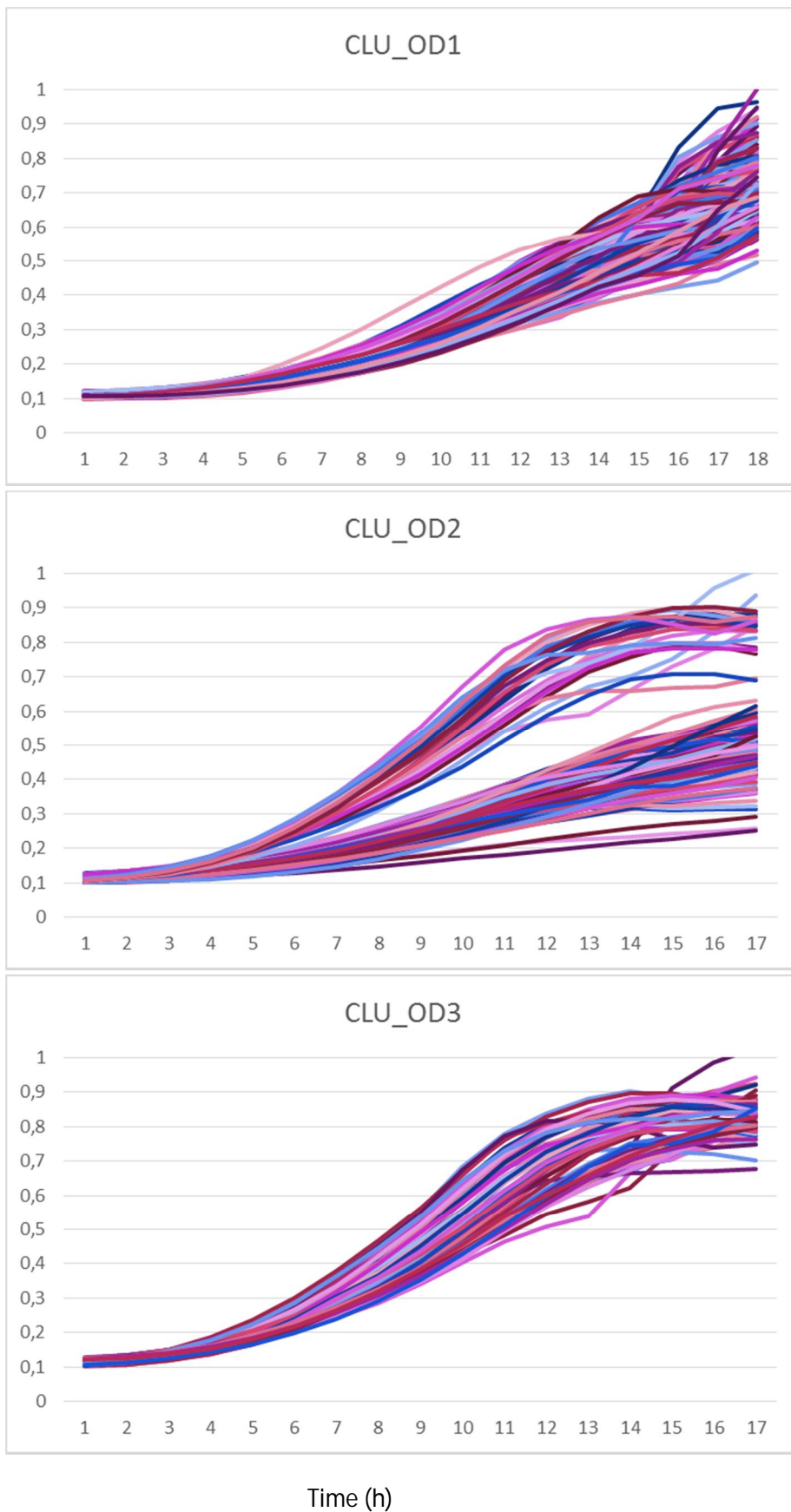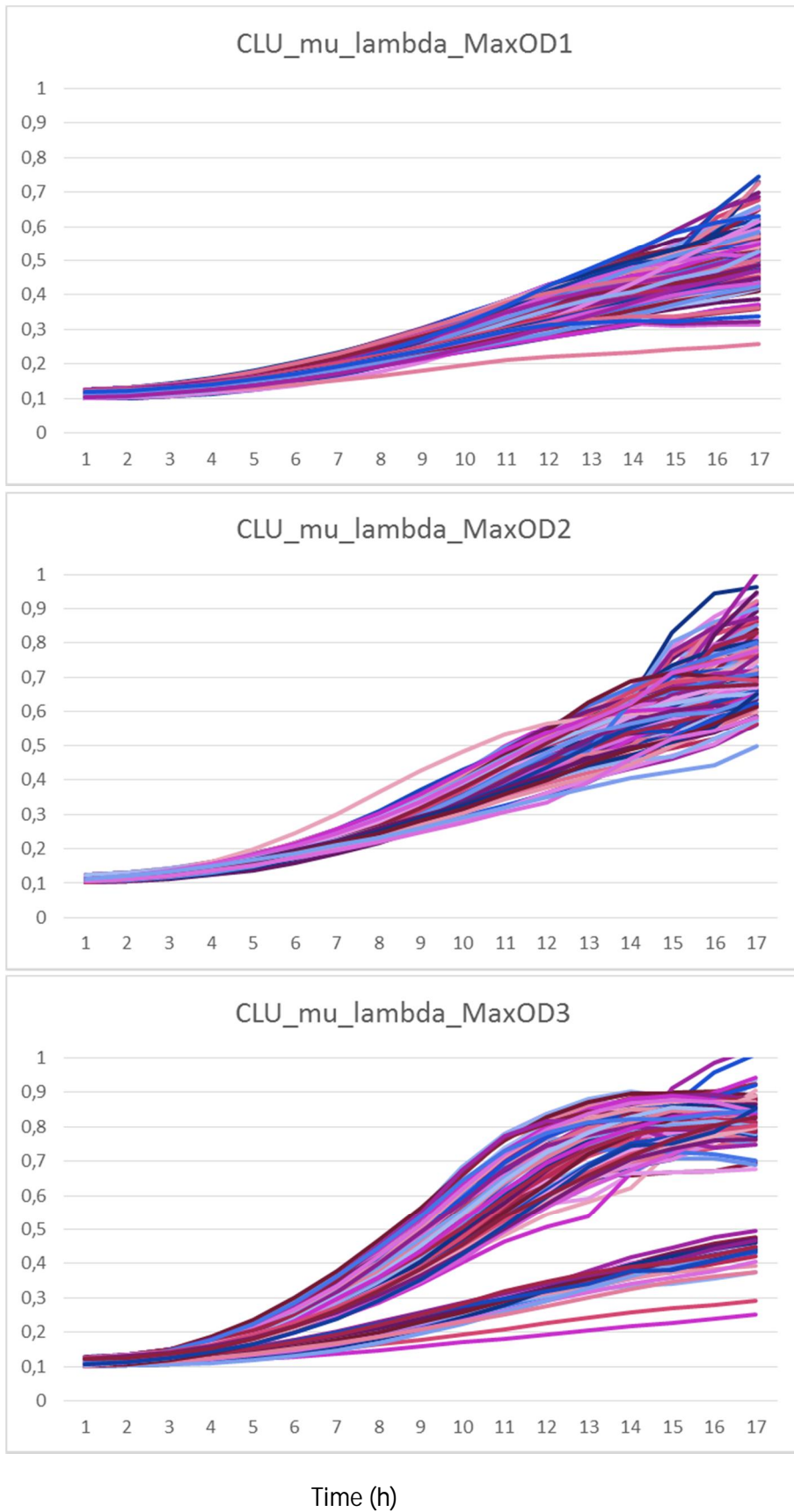
Supplemental Figure S12. Distribution of *Listeria monocytogenes* strains (n = 388) into growth clusters determined by $OD_{600}$ as clustering variable (alternative clustering outcome 1).
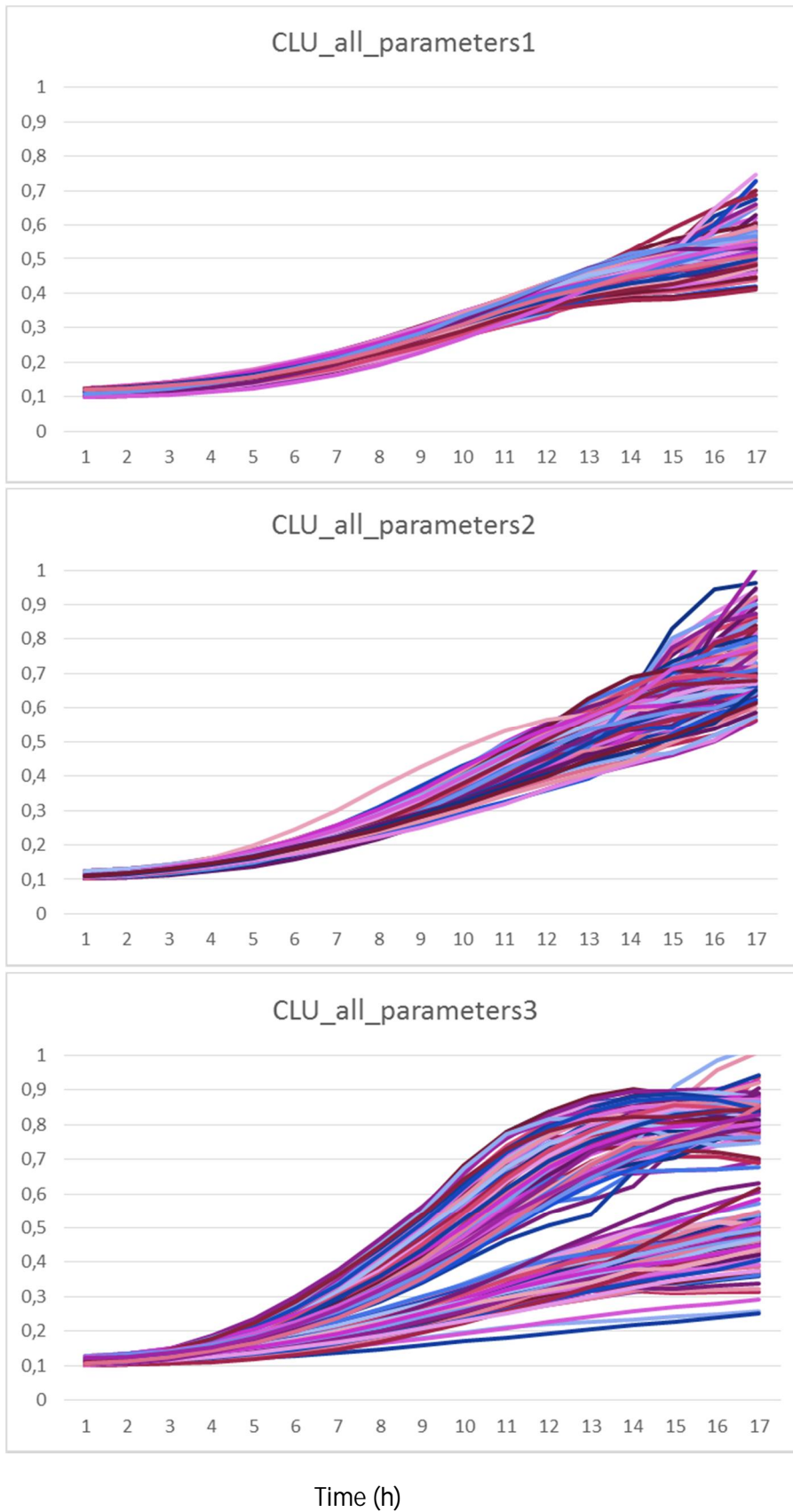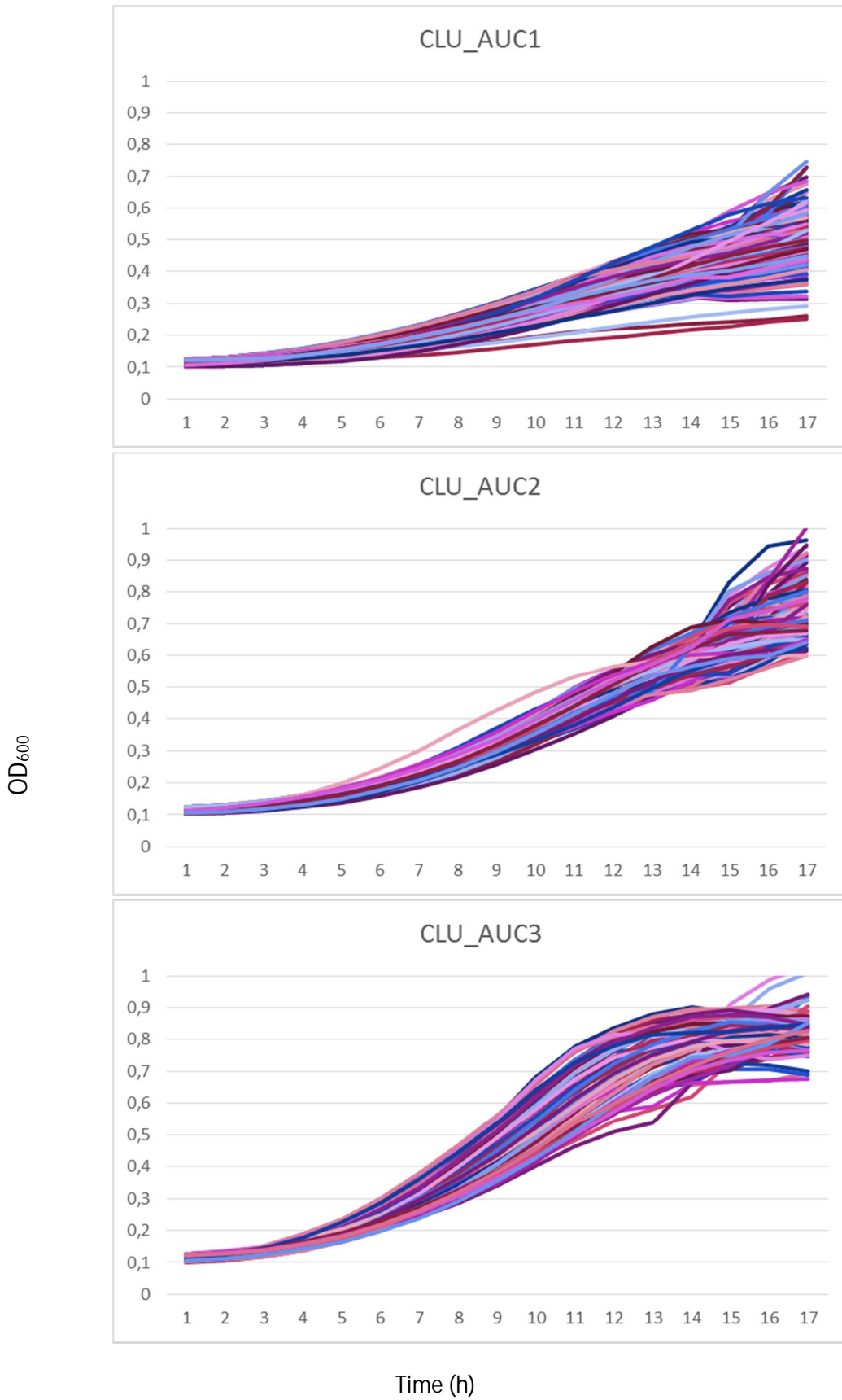
Supplemental Figure S13. Distribution of *Listeria monocytogenes* strains (n = 388) into growth clusters determined by lag phase (λ), growth rate (μ), maximum growth density (MaxOD), and area under the curve (AUC) as clustering variables (alternative clustering outcome 2).

Supplemental Figure S14. Distribution of *Listeria monocytogenes* strains (n = 388) into growth clusters determined by lag phase (λ), growth rate (μ), and maximum growth density (MaxOD) as clustering variables (alternative clustering outcome 3).

Supplemental Figure S15. Distribution of *Listeria monocytogenes* strains (n = 388) into growth clusters determined by area under the curve (AUC) as clustering variable (alternative clustering outcome 4).