HOST-DIALOG

SOP-HD-001 · V1.1

Day 0 — Bench Card

PRINT · LAMINATE · BSC-SIDE

Strain: USA300 JE2
Medium: CDM (Hussain)
Volume: 100 mL master · 10 mL × n daughters

PRE-FLIGHT (T−30)

☐ incubator @ 37 °C
☐ CDM pre-warmed
☐ tracer dilutions fresh
☐ drug stocks thawed
☐ flasks labelled
☐ tubes on ice
☐ TCA on ice
☐ 90 °C bath ON
☐ acid-fix buffer cold
☐ scintillation vials lined up
☐ timer ready
☐ log sheet open

TIME	CLOCK	ARM	ACTION	VOLUME / SPEC
T−90	07:30 → 08:00	**PREP**	Dilute preculture into master flask. From overnight (OD ≈ 3) into pre-warmed CDM to OD_{600} = 0.05.	100 mL · 500 mL
T−60	08:30	**PREP**	First OD_{600} check. Begin 15-min monitoring. Expect OD ≈ 0.10–0.15. Record. If far off, flag and continue.	200 µL aliquot
T−15	09:15	**PREP**	Pre-warm daughter flasks. Final label check. Verify date · strain · drug · arm · replicate on every flask. Confirm drug working dilutions ready.	n × 50 mL flask
T 0	09:30	**SPLIT + DRUG**	SPLIT MASTER · APPLY DRUGS SIMULTANEOUSLY. 10 mL master into each daughter via 25 mL serological. Add drug/vehicle within 30 s of split. **Start main timer.**	10 mL/flask + d
T 0	09:30 (master)	**CLEAN**	Sample T0 from MASTER (pre-split). This is the shared baseline for all RNA arms. Pellet, decant, snap freeze.	1.5 mL × 2 (AU
T 15	09:45	**CLEAN**	Sample T15 per condition. AU sample → cold acid-fix buffer (0.3 M NaOAc pH 4.5). sRNA sample → TRIzol LS direct on pellet.	1.5 mL × 2
T 30	10:00	**HOT**	Add ^{3}H tracer to hot daughter flasks. 100 µL tracer working stock per 10 mL → final 0.05 µCi/mL. Record exact addition time per flask. Return to shaker.	100 µL ^{3}H stock
T 30	10:00	**CLEAN**	Sample T30 per condition. As T15. Speed matters — pellet to ice within 60 s, freezer within 10 min.	1.5 mL × 2
T 45	10:15	**HOT**	PULSE END. Quench + TCA-precipitate. 3 × 1 mL aliquots → 1 mL ice-cold 10 % TCA. Mix, ice 20 min.	3 × 1 mL → TCA
T 45	10:15	**CLEAN**	Sample T45 per condition. Mid-pulse equivalent on the regulatory side.	1.5 mL × 2
T 60	10:30	**CLEAN**	Sample T60 per condition. Post-pulse continuing-effect window.	1.5 mL × 2
T 45+	10:35 →	**HOT**	Filter onto GF/C · hot-TCA wash. 3 × 5 mL ice-cold 10 % TCA wash on manifold. Then 90 °C in 5 % TCA × 20 min. Cold 5 % TCA wash. 2 × cold EtOH wash. Air-dry.	GF/C 25 mm
T 120	11:30	**CLEAN**	Sample T120 per condition. Last clean-arm sample. Late regulatory response window.	1.5 mL × 2
T ~150	12:00 →	**HOT**	Vials + 10 mL Ultima Gold · count. Tri-Carb, ^{3}H channel (or dual $^{3}H/^{14}C$), 2 min/vial. DPM into log sheet then into shared drive.	10 mL cocktail
T ~180	13:00 →	**CLEAN**	Acid-fix arm: phenol extract · ethanol pp. Process all AU samples within 4 h of collection. Resuspend in cold acid buffer. Hold at 4 °C max overnight.	200 µL aq.

ACCEPTANCE GATES · STOP AND RERUN IF ANY FAIL

- **Master OD at split:** `0.48–0.52`
- **Timing deviation per time-point:** < `2 min`
- **Vehicle hot-arm CV:** < `15 %`
- **CAM hot-arm reduction:** > `70 %` all tracers
- **Sample → ice:** < `60 s` from decant
- **Sample → −80 °C:** < `10 min` from collection

THE 5-SECOND MECHANISM REMINDER

- **S. aureus has no AsnRS.** Free Asn cannot rescue a GatCAB block.
- **^{3}H-Asp is the cleaner probe** — bypasses the AnsA deamination delay.
- **The diagnostic ratio:** `[³H-Asn]/[³H-Leu]` drops < `0.6` = GatCAB-specific.
- **If all four tracers drop equally** = generic translation hit, not the mechanism.
- **Acid-urea: the headline.** Asp-tRNA-Asn band rising = direct biochemical proof.
- **Speed kills aminoacyl bonds.** Cold + acid + fast, always.

RAD **^{3}H is a pure beta emitter — internal contamination is the hazard, not external dose.** Double-glove. Bench paper down. Pipette below surface. Swipe-test hands and bench BEFORE leaving the hot zone. No food, drink, cosmetics anywhere in the radiation area. Spill > 1 MBq → call radiation officer within 24 h.

HOST-DIALOG · SOP-HD-001 v1.1 · April 2026 · Karlsen / Bollvåg et al.

"Cold, acid, fast — the only theology in this lab."