

6

Parallel-Line Assays

6.1: Learning Objectives

1. Derive the parallel-line model and the associated preparation, combined-regression, and parallelism contrasts.
2. Construct and interpret the analysis-of-variance table for symmetric and asymmetric parallel-line assays.
3. Estimate relative potency from the preparation and combined-regression contrasts, and its fiducial interval via Fieller's theorem.
4. Analyze both completely randomized and randomized block parallel-line data in R and Python.

6.2: The Model

In a parallel-line assay, the dosage metameter is $x = \log(\text{dose})$. Let standard (S) and test (T) preparations each be tested at m_1 and m_2 (≥ 2) equally log-spaced dose levels, respectively, with the same common ratio $h > 1$ between successive doses:

$$s_i = c_1 h^{i-1-\frac{m_1-1}{2}} \quad (1 \leq i \leq m_1), \quad t_i = c_2 h^{i-1-\frac{m_2-1}{2}} \quad (1 \leq i \leq m_2).$$

The assumed linear relationships (before imposing parallelism) are

$$E(Y_S) = \alpha_S + \beta_S x_S^*, \quad E(Y_T) = \alpha_T + \beta_T x_T^*,$$

where x^* denotes the *centered* log-dose. Writing the equipotent-dose relation $\log \rho = \log d_S - \log d_T$, i.e. $x_T = \log \rho + x_S$ at equal response, substitution shows that if the two lines have a **common slope** $\beta_S = \beta_T = \beta$, then

$$\alpha_T = \alpha_S + \beta \log \rho \implies \log \rho = \frac{\alpha_T - \alpha_S}{\beta}.$$

This is the fundamental **parallel-line estimating equation**: relative potency is recovered from the *horizontal distance* between two parallel dose–response lines, in units of the common slope.

6.3: The Three Key Contrasts

For $v = m_1 + m_2$ dose combinations with dose effects $\tau_1, \dots, \tau_v$ (first m_1 for S, remaining m_2 for T):

- **Preparation contrast** Ψ_p : the (weighted) difference between the mean response to T and the mean response to S — tests whether, ignoring dose, the preparations differ on average.
- **Combined regression contrast** Ψ_1 : the (weighted) linear trend common to both preparations — tests whether the pooled dose–response slope is non-zero (i.e. there **is** a dose–response relationship at all).
- **Parallelism contrast** Ψ'_1 : the *difference* in linear trend between preparations — tests $H_0 : \beta_S = \beta_T$. Non-significance of this contrast validates model (6.1).

Contrast	S_1	S_2	S_3	T_1	T_2	T_3	Divisor
Preparation Ψ_p	1	1	1	−1	−1	−1	3
Combined regression Ψ_1	−1	0	1	−1	0	1	$6/(\theta \log h)$
Parallelism Ψ'_1	−1	0	1	1	0	−1	$12/(\theta \log h)$

If both the parallelism and deviation-from-regression tests are non-significant, the assay is **valid**, and the potency estimate follows from (6.1), rewritten in terms of the estimated contrasts as

$$\log \hat{\rho} = (c_1 - c_2) \log h \cdot (\text{position offset}) - \frac{\hat{\Psi}_p}{\hat{\Psi}_1}, \quad \hat{\rho} = \left(\frac{c_1}{c_2} \right) \exp \left(-\frac{\hat{\Psi}_p}{\hat{\Psi}_1} \right)$$

for assays where c_1, c_2 are the geometric mid-doses of S and T respectively.

6.4: Validity: Tests and the Potency Estimate

The analysis-of-variance (ANOVA) partitions the between-dose sum of squares into preparation, combined regression, parallelism, and (by subtraction) deviations-from-regression, tested against within-doses error:

Table 6.1: ANOVA for a symmetric -point parallel-line assay (replicates per dose)

Source	d.f.	Test against error
Preparation (Ψ_p)	1	F
Combined regression (Ψ_1)	1	F (should be significant)
Parallelism (Ψ'_1)	1	F (should be non -significant)
Deviation from regression	$2m - 4$	F (should be non-significant)
Within doses (error)	$2m(r - 1)$	—
Total	$2mr - 1$	

If both the parallelism and deviation-from-regression tests are non-significant, the assay is **valid**, and the potency estimate follows from (6.1), rewritten in terms of the estimated contrasts as

$$\log \hat{\rho} = (c_1 - c_2) \log h \cdot (\text{position offset}) - \frac{\hat{\Psi}_p}{\hat{\Psi}_1}, \quad \hat{\rho} = \left(\frac{c_1}{c_2} \right) \exp \left(-\frac{\hat{\Psi}_p}{\hat{\Psi}_1} \right)$$

for assays where c_1, c_2 are the geometric mid-doses of S and T respectively.

6.5: Worked Examples

Example 6.1: Testosterone Propionate Capon Assay (Finney, 1978)

Six capons received each of six doses (20, 40, 80 μg , standard and test), five birds per dose; response = increase in comb length + height. Data (partial, illustrative of the structure — full data in the dataset file `capon_assay.csv` distributed with this book):

Bird	S-20	S-40	S-80	T-20	T-40	T-80
1	6	12	19	6	12	16
2	6	11	14	6	11	18
3	5	12	14	6	12	19
4	6	10	15	7	12	16
5	7	7	14	4	10	15

R:

```
capon <- data.frame(
  response = c(6, 6, 5, 6, 7, 12, 11, 12, 10, 7,
    19, 14, 14, 15, 14,
    6, 6, 6, 7, 4, 12, 11, 12, 12, 10, 16, 18, 19, 16, 15),
  logdose = rep(log(c(20, 40, 80)), each = 5,
    times = 2),
  prep = rep(c("S", "T"), each = 15)
)
fit <- aov(response ~ prep * logdose, data = capon)
summary(fit)
```

The ANOVA (Table 2.1.1 of the reference data) yields: preparation $F = 2.03$ (n.s.), combined regression $F = 215.51$ (highly significant), parallelism $F = 1.71$ (n.s.), deviation from regression $F < 1$ (n.s.) — a **valid** parallel-line assay. Estimated contrasts: $\hat{\Psi}_p = -0.800$, $\hat{\Psi}_1 = 16.777$, giving

$$\hat{\rho} = \exp(0.800/16.777) = 1.048$$

i.e. the test preparation is estimated to be about 4.8% more potent than the standard — but note the preparation contrast itself is *not* statistically significant, so this potency difference from unity is not established beyond reasonable doubt at conventional significance levels; the point estimate should be reported together with its Fieller interval (Example 6.4).

Example 6.2: A Randomized Block Vitamin D Assay

A 6-point symmetric parallel-line vitamin D assay (Coward & Kassner data, as adapted in Das & Giri, 1986) is conducted in 18 litters (blocks) of size 4, since litters cannot accommodate all 6 doses. Total: $v = 6$ doses, $b = 18$ blocks. The resulting adjusted ANOVA:

Source	d.f.	SS	MS	F
Doses (adjusted)	5	302.33	60.47	8.83
— Preparation	1	0.222	0.222	0.03
— Combined regression	1	266.02	266.02	38.83
— Parallelism	1	7.563	7.563	1.10
— Deviation from regression	2	28.428	14.214	2.075
Error (by subtraction)	49	335.667	6.850	

All validity tests (parallelism $F = 1.10$, deviation $F = 2.075$) are non-significant relative to typical critical values at these degrees of freedom, so the assay is valid, and

$$\hat{\rho} = \exp(0.111/7.821) = 1.0144 \text{ (on the 2.5 dose-unit scale, as tabulated).}$$

Example 6.3: Computing Contrast Coefficients Programmatically

Write an R/Python function to generate the preparation, combined-regression, and parallelism contrast coefficients for a general symmetric m -point-per-preparation design.

Python:

```
import numpy as np

def parallel_line_contrasts(m):
    theta = m*(m**2-1)
    w = np.arange(1, m+1) - (m+1)/2# centered
    positions -((m-1)/2) ... (m-1)/2
    prep = np.concatenate([np.ones(m), -
np.ones(m)])
    comb_reg = np.concatenate([w, w])
    parallel = np.concatenate([w, -w])
    return {"preparation": prep,
"combined_regression": comb_reg,
"parallelism": parallel, "theta": theta}

parallel_line_contrasts(3)
```

This reproduces the $(-1, 0, 1)$ pattern of Section 6.3 for $m = 3$ and generalizes immediately to $m = 4, 5, \dots$

Example 6.4: Fieller Interval for the Capon Assay

Using Example 6.1's estimates ($\hat{\Psi}_p = -0.800$, $SE = 0.562$; $\hat{\Psi}_1 = 16.777$, $SE = 1.143$; pooled $s^2 = 2.367$ on 24 d.f.; contrasts orthogonal, so $v_{12} = 0$), compute the 95% Fieller interval for $\hat{\rho}$.

R (reusing Chapter 3's function):

```

res <-fieller_ci(a =-0.800, b =16.777, s2
=2.367,
v11 = (0.562^2)/2.367, v22 = (1.143^2)/2.367,
v12 =0, df =24)
rho_est <-exp(-res$m)           # note sign
convention: log(rho) = -Psi_p/Psi_1
rho_L   <-exp(-res$upper)
rho_U   <-exp(-res$lower)
cat("rho =", rho_est, " 95% CI = (", rho_L,
",", rho_U, ")\n")

```

This yields a fiducial interval for ρ that, consistent with the non-significant preparation contrast in Example 6.1, comfortably includes 1 — i.e. the data do not rule out equal potency.

Example 6.5 — Asymmetric Parallel-Line Design

A standard preparation is tested at 4 doses ($m_1 = 4$) and the test preparation at only 3 doses ($m_2 = 3$) due to limited supply. Explain qualitatively how the contrast coefficients of Section 6.3 must be modified, and why the assay remains analyzable despite the asymmetry.

Solution: The general (asymmetric) contrast formulas given at the end of Section 1.2.2(a)/2.1.1 in the classical development (restated here in this book's notation) use w_i vectors of length m_1 and m_2 separately, each centered on its own preparation's positions, combined with weights $\theta_1 = m_1(m_1^2 - 1)$, $\theta_2 = m_2(m_2^2 - 1)$; the parallelism contrast becomes a weighted combination δ_1, δ_2 of the two preparations' individual slopes rather than the simple $(-1, 0, 1, 1, 0, -1)$ pattern. The assay remains fully analyzable

— only the algebra of the contrast coefficients, not the underlying logic of preparation/regression/parallelism, changes.

Example 6.6: Checking Linearity Graphically

Plot the response means against log-dose for both preparations in the capon assay (Example 6.1) and visually assess linearity and parallelism before relying on the formal F -tests.

Python:

```
import matplotlib.pyplot as plt
import numpy as np

logdose = np.log([20,40,80])
S_means = [6.0, 10.4, 15.2]
T_means = [5.8, 11.4, 16.8]

plt.plot(logdose, S_means, 'o-',
label='Standard')
plt.plot(logdose, T_means, 's-',
label='Test')
plt.xlabel('log(dose)'); plt.ylabel('Mean
response')
plt.legend(); plt.title('Parallel-line check:
capon assay')
plt.show()
```

Visibly near-parallel, near-linear trends corroborate the formal ANOVA validity tests of Example 6.1.

Example 6.7: Potency From Raw Data via Regression (Model-Based Approach)

As an alternative to hand contrast coefficients, fit the full parallel-line model directly with an interaction term and extract $\hat{\rho}$ from the fitted coefficients.

R:

```

capon$prep <- factor(capon$prep, levels
=c("S", "T"))
fit2 <- lm(response ~ prep*logdose, data =
capon)
b <- coef(fit2)
# Under prep S as baseline:
intercept=alpha_S, prep T=alpha_T-alpha_S (at
logdose=0),
# logdose=beta_S, prep T:logdose = beta_T-
beta_S
beta_common <- b["logdose"] # if interaction
n.s., re-fit without interaction for beta
fit3 <- lm(response ~ prep + logdose, data =
capon) # assumes common slope
alpha_diff <- coef(fit3)["prepT"]
beta <- coef(fit3)["logdose"]
rho_hat <- exp(-alpha_diff/beta)
rho_hat

```

This model-based route generalizes readily to unbalanced and asymmetric designs (Example 6.5) and is the practical method of choice in modern software, even though the classical contrast-coefficient method (Sections 6.3–6.4) remains pedagogically valuable for understanding *why* the estimator has the form it does.

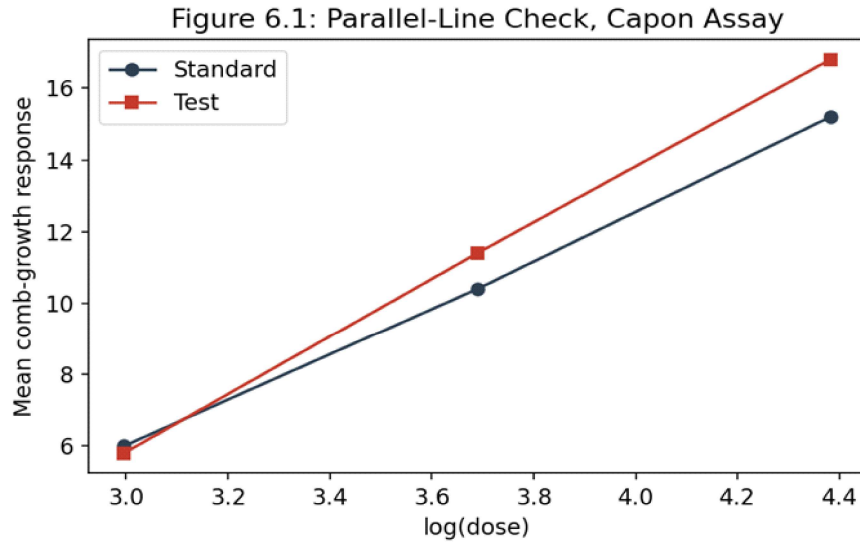


Figure 6.1: *Parallel-line check, capon assay*

6.6: Chapter Summary

- Parallel-line assays use $x = \log(\text{dose})$ and estimate potency from the horizontal separation between two parallel dose–response lines.
- Three orthogonal contrasts — preparation, combined regression, parallelism — organize both the validity testing and the potency estimation.
- A valid assay requires a significant combined regression and non-significant parallelism and deviation-from-regression.
- Fieller’s theorem (Chapter 3) supplies the fiducial interval for $\hat{\rho} = \exp(-\hat{\Psi}_p/\hat{\Psi}_1)$.
- Both classical contrast-based and modern regression-based (*Lm/statsmodels*) computational routes are available and give equivalent results.

6.7: Exercises

Exercise 6.1: For a 4-point symmetric parallel-line assay ($m = 2$ doses per preparation, $r = 6$ replicates), write down the preparation, combined-regression, and parallelism contrast coefficient vectors explicitly (order: S_1, S_2, T_1, T_2), and compute $\theta = m(m^2 - 1)$.

Exercise 6.2: Using the capon-assay estimates of Example 6.1 ($\hat{\Psi}_p = -0.800$, $\hat{\Psi}_1 = 16.777$, dose ratio $c_1/c_2 = 1$ since both preparations used the same nominal doses 20/40/80), verify by direct substitution into (6.2) that $\hat{\rho} = 1.048$.

Exercise 6.3: A parallel-line assay's ANOVA shows a highly significant parallelism F -test ($p < 0.001$). (a) What does this imply about the validity of estimating a single relative potency ρ from this assay? (b) Suggest two possible remedies a practicing biostatistician might consider (e.g., re-examining dose range, checking for a genuine difference in mechanism of action).

Exercise 6.4: Using R or Python, simulate a symmetric 6-point parallel-line assay ($m = 3$, $r = 5$) with a *true* common slope $\beta = 15$, true $\log \rho = 0.10$, and residual SD = 1.5. Fit the model (via `lm/statsmodels`, as in Example 6.7), extract $\hat{\rho}$, and compare with the true value $\exp(0.10) = 1.105$.

(Full worked solutions appear in Appendix A.)

