Stability experiments, modeling and shelf life estimation. In use and accelerated stability testing.

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Basic concepts and terminology

- **Stability**: the ability of a product to maintain its performance characteristics consistent over time.
  - It is expressed as time interval that a product characteristics remain within the specification of the manufacturer

- **Shelf-Life**: time interval that a product is stable at the recommended storage conditions
**Degradation**: change (loss of activity or/and decrease of performance) of the characteristics of a product as it ages.

**Degradation rate**: the amount of change in a unit of time. Degradation rate can be constant or variable and depends on ambient conditions.

**Measurand Drift**: the observed change under specific conditions.
- Can be quantified in terms of absolute or relative change
- Usually compared to time 0 (defined)
- Estimated by statistical modeling
Factors that define stability

- *Performance metrics*: metrics that are most likely to reveal potentially important changes (quality, safety, efficacy)

- *Stability claims*
  Are usually expressed in terms of time (e.g. product should performed within the intended specifications for 100 days) and storage conditions (e.g. stored at room temperature)

- *Stability Specs*
  Are usually expressed in terms of a allowable drift (tolerable change) from time zero (e.g. product should change no more than 10%)

- *Statistical confidence and power*
Stability tests to assess shelf-life

- **Real-time tests**: product is stored in normal storage conditions and monitored for a period of time.
- **Accelerated tests**: product is stored at elevated stress conditions (temperature, humidity, etc.) and monitored.

- In-Use Stability studies (open container, onboard stability)

- Transport studies (stress studies, simulations....)
Degradation reaction

- **Zero order** reaction involves one kind of molecules and the degradation process does not depend on the number of remaining molecules.

- **First order** reaction involves one kind of molecules and the degradation process is proportional to the number of remaining molecules.

- **Second and higher orders** reactions involve multiple interactions of two or more kinds of molecules and are characteristic for most biological materials that consist of large and complex molecular structures.
Statistical models for stability testing

- Zero order kinetics reaction
  \[ Y = \alpha - \delta t + \varepsilon \]

- First order kinetics reaction
  \[ Y = \alpha \exp(-\delta t) + \varepsilon \]

  - \( Y \) is the observed testing result
  - \( \alpha \) is the performance of each lot at time zero
  - \( \delta \) is the degradation rate
  - \( t \) is time
  - \( \varepsilon \) is the random experimental error
Experimental designs, testing intervals, and sample size

General

- Design depend on the product that is tested
- The most common design is testing several random replicates at each time points
- Designs that include other factors in stability testing (container size, lots, etc) can be implemented
Experimental designs, testing intervals, and sample size

Number of time points

- There is no sound statistical approach to determine the number of testing points.
- Need to have as many time points as to capture the degradation trend
  - Degradation pattern (linear vs. more complicated functions)
  - Degradation rate
  - Temperature
- Purpose of the test (determine shelf life, validation, confirmatory, etc.)
- Resources
Experimental designs, testing intervals, and sample size

Sample Size

- Determine error based on repeatability and reproducibility

\[ \sigma^2_Y = \frac{r \sigma^2_{\text{repro}} + \sigma^2_{\text{repea}}}{n \ r} \]

- \( \sigma^2_{\text{repro}} \) is the reproducibility variance
- \( \sigma^2_{\text{repea}} \) is the repeatability variance
- \( r \) is number of replicates for each time point
- \( n \) is number of time points

- Tolerable Difference

- Power of the test

\[ \text{Power} = 1 - \text{prob}[ t(df, \delta) \geq t(df, \alpha)] \]
Experimental designs, testing intervals, and sample size

Sample Size

- Simulation for:
  - Repeatability CV = 3%
  - Reproducibility CV = 2%
  - $\alpha = 0.05$
  - Tolerable difference = 5%

![Power for n=10 and different replicates](image)

- Linear
- Exponential
- Polynomial
- Exponential_S
Real time stability statistics

- Estimate Intercept and Degradations rate
- Estimate measurand drift at target shelf life
- Estimate 95% confidence one-sided interval of the drift
- Compare the upper limits of the drift confidence interval to the acceptance criterion (tolerable drift)
- Product meets target shelf life when drift (CI) < acceptance criterion
- Short date product stability when drift (CI) > acceptance criterion
  - Predict shelf life (time) from the statistical degradation model
Graphical presentation of real time stability statistics

- Measurand
- Degradation line
- Limits

Tolerable Drift (Spec) = 0.9
Stability target = 365 days

365 days 372 days
Measurand drift

Predicted Stability
Isochronous designs for stability

- Time point to time point variability is usually large
  - Day-to-day measurement variability
  - Calibration of measurement devices
  - Need to increase number of time points

- Product tolerate for a freeze and thaw cycle.

- Remove vials at different time points and freeze (or store in conditions when no degradation occurs)

- Test all vials at the same time at the end

- Same statistical modelling and estimation of drifts
Closed & Open container stability

- **Evaluation of Stability of CD4 marker**

- **Experimental design**
  - Seven time points during the target closed container stability period (154 days ≈ 5 months).
  - 5-10 replicates each time point.
  - Four time points during the target open container stability period (35 days ≈ 1 months).
  - 5 replicates each time point.
Closed & Open container stability

![Graph showing CD4 cells over days for closed and open containers with degradation trends.](image-url)
**Statistical Model**

- \( Y_i = \alpha_0 + \alpha_1 t_{ct} + \beta_1 t_{oi} + \delta_i \)
  - \( \alpha_0 \) - the intercept (response at time zero)
  - \( \alpha_1 \) - degradation rate of closed container
  - \( t_{ct} \) - time of closed container
  - \( \beta_1 \) - degradation rate of open container
  - \( t_{oi} \) - time of open container
  - \( \delta_i \) - experimental error
<table>
<thead>
<tr>
<th>Marker</th>
<th>Container</th>
<th>Parameter</th>
<th>Estimate</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 cells/uL</td>
<td>Closed</td>
<td>Intercept</td>
<td>715.5</td>
<td>707.8</td>
<td>723.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Degradation Rate</td>
<td>-0.359</td>
<td>-0.463</td>
<td>-0.254</td>
</tr>
<tr>
<td></td>
<td>Open</td>
<td>Intercept</td>
<td>660.3</td>
<td>645.0</td>
<td>675.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Degradation Rate</td>
<td>-1.112</td>
<td>-1.735</td>
<td>-0.490</td>
</tr>
</tbody>
</table>
Closed & Open container stability

Closed Drift

Open Drift

Total Drift

Days

0 20 40 60 80 100 120 140 160 180 200
Estimation of Drifts

- Drift at target time $t_{ct}$ of open container and confidence interval
  \[ D_c = \alpha_1 t_{ct} \]
  \[ \text{Se}(D_c) = \text{Se}(\alpha_1) t_{ct} \]
  \[ D_c \pm 2 \times \text{Se}(D_c) \]

- Drift at target time $t_{ot}$ of open container and confidence interval
  \[ D_o = \beta_1 t_{ot} \]
  \[ \text{Se}(D_o) = \text{Se}(\beta_1) t_{ot} \]
  \[ D_o \pm 2 \times \text{Se}(D_o) \]

- Stability total drift
  \[ D_s = D_c + D_o \]
  \[ \text{Se}(D_s) = \sqrt{\frac{\text{Se}(D_c)^2 \times df_c + \text{Se}(D_o)^2 \times df_o}{df_c + df_o}} \]
  \[ D_s \pm 2 \times \text{Se}(D_s) \]
## Drifts

<table>
<thead>
<tr>
<th>Marker</th>
<th>Container</th>
<th>Drift</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 cells/uL</td>
<td>Closed = 154 Days</td>
<td>-55</td>
<td>-71</td>
<td>-39</td>
</tr>
<tr>
<td></td>
<td>Open = 30 Days</td>
<td>-33</td>
<td>-52</td>
<td>-15</td>
</tr>
<tr>
<td></td>
<td>Total = 184 Days</td>
<td>-89</td>
<td>-113</td>
<td>-64</td>
</tr>
</tbody>
</table>
Accelerated stability

- Accelerate the degradation by using elevated conditions
- Define elevated conditions
- Design stability testing
- Shelf-life is assessed in two steps
  - Estimation of degradation rates and stability at each elevated condition
  - Prediction of degradation rate and shelf-life based on a known relationship between the accelerated factor and the degradation rates
Statistical models for stability testing

**Temperature**

- **Arrhenius equation**

\[ \delta = A \exp \left( -\frac{E_a}{RT} \right) \]

\( \delta \) is the degradation rate, \( A \) is an Arrhenius factor, \( E_a \) is the activation energy (kcal mol\(^{-1}\)), \( R = 0.00199 \) (kcal mol\(^{-1}\) K\(^{-1}\)) is the gas constant, and \( T \) is temperature in Kelvin (K) = °C + 273.

- **Acceleration factor**

\[ \lambda = \exp \left[ \frac{E_a}{0.00199} \left( \frac{1}{T_s} - \frac{1}{T_e} \right) \right] \]

\( E_a \) is the activation energy. This is equal to the energy barrier that must be exceeded for the degradation reaction to occur. \( E_a \) is a property of the product, \( T_s \) is the storage temperature, \( T_e \) is the elevated temperature.
Statistical models for stability testing
Accelerated degradation

- Zero order kinetics reaction
  \[ Y = \alpha - \delta \lambda t + \varepsilon \]

- First order kinetics reaction
  \[ Y = \alpha \exp(-\delta \lambda t) + \varepsilon \]

- \( Y \) is the observed testing result
- \( \alpha \) is the performance of each lot at time zero
- \( \delta \) is the degradation rate
- \( \lambda \) is the acceleration factor
- \( t \) is time
- \( \varepsilon \) is the random experimental error
Statistical models for stability testing

- **Product**
  - COULTER CLENZ®. This is a cleaning agent which is aspirated during the shutdown cycle of a Beckman Coulter hematology instrument.
  - Recommended storage temperature is 25°C
  - Cut-off for stability is 70% of the original performance

- **Accelerated test**
  - Three elevated temperatures, 40°C, 45°C, 50°C
  - Three lots
  - Number of replicates and time points were predetermined based on an experimental design that controls and reduces random variability
Statistical models for stability testing

Degradation trends at elevated temperatures

- Stability Cut-off
- 55°C
- 45°C
- 40°C

Days

Days

Days

Days
### Statistical models for stability testing

#### Estimates of stability

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Days</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>55°C</td>
<td>8.1</td>
<td>6.9</td>
<td>9.3</td>
</tr>
<tr>
<td>45°C</td>
<td>30.1</td>
<td>28.0</td>
<td>32.3</td>
</tr>
<tr>
<td>40°C</td>
<td>73.2</td>
<td>61.3</td>
<td>85.1</td>
</tr>
<tr>
<td>25°C</td>
<td>616</td>
<td>492</td>
<td>741</td>
</tr>
</tbody>
</table>
### Statistical models for stability testing

#### Estimates of parameters

<table>
<thead>
<tr>
<th>Fixed parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.999</td>
<td>0.0046</td>
<td>0.990</td>
<td>1.008</td>
</tr>
<tr>
<td>Degradation rate</td>
<td>0.0006</td>
<td>0.00006</td>
<td>0.0005</td>
<td>0.0007</td>
</tr>
<tr>
<td>Activation energy</td>
<td>28.2</td>
<td>0.79</td>
<td>26.6</td>
<td>29.7</td>
</tr>
</tbody>
</table>
Statistical models for stability testing

Degradation at room temperature

Days

Stability Cut-off

25

Lower

Upper
Second or higher -order kinetics reaction

Product
- HmX PAK reagent system used for enumerating WBC 5-part differential on a COULTER® HmX Analyzer
- Recommended storage temperature is 25°C
- Cut-off for stability is 0.63 of the original performance

Accelerated test
- five elevated temperatures, 60°C, 55°C, 50°C, 45°C, 40°C
- Five fresh in-house donor blood specimens in duplicate
Degradation at elevated temperatures

The graph illustrates the degradation of a material under different temperatures. The x-axis represents days, and the y-axis represents a degradation parameter. The graph shows the lag phase and degradation phase for different temperature conditions: 60°C (triangle), 55°C (square), 50°C (dashed line), 45°C (plus symbol), and 40°C (times symbol). The outside specification is marked by a red arrow indicating when the degradation exceeds the specification limit.
Degradation

- Product degrades in two phases
  - Lag phase: degradation is not experimentally detectable in most of the temperatures
  - Degradation phase: significant degradation
Arrhenius requirements

- The assumption of zero- or first-order kinetics reaction is violated. There is indication of second or higher -order kinetics reaction

- The same model is used to fit the degradation patterns at each temperature

- Need to test - Linear relationship between degradation rate (LOG transformation) and temperature (inverse)
Degradation model

- Elevated temperatures [1]
  \[ Y = \beta_0 + (\beta_1 + \beta_2 t) t + \varepsilon \]

- Storage temperature [2]
  \[ Y = \beta_0 + (\beta_1 + \beta_2 t) t \lambda + \varepsilon \]

- Degradation rate is a function of time
  \[ \delta = \beta_1 + \beta_2 t \]

- Stability
  \[ t_{\text{Stab}} = \frac{(-\beta_1 - \sqrt{\beta_1^2 - 4\beta_2 (\beta_0 - Y_{\text{Crit}})})}{2\beta_2} \]
Estimates of stability

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Lag Phase (Days)</th>
<th>Stability (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated</td>
<td>Lower</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>55</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>45</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>40</td>
<td>28</td>
<td>17</td>
</tr>
</tbody>
</table>
Linear relationship

- Calculate degradation rate at each elevated temperature ($\delta_{[1]}$) at $t_{Stab}$ using [1]
- Calculate degradation rate at each elevated temperature ($\delta_{[2]}$) at $t_{Stab}$ using [2]
- Variances of degradation rates

\[ s^2_\beta_1 + t^2 s^2_\beta_2 + 2t s_{\beta_1,\beta_2} \]

- Calculate the pooled variance, $s^2_\delta$ from the variances of the degradation rates

\[ X^2 = \sum \left( \frac{\hat{\delta}_{[1]} - \hat{\delta}_{[2]}}{s^2_\delta} \right)^2 \] is distributed as Chi-square
Linear relationship

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Degradation rates at $t_{\text{Stab}}$</th>
<th>$s^2_\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta_{[1]}$</td>
<td>$\delta_{[2]}$</td>
</tr>
<tr>
<td>60</td>
<td>-0.0551506</td>
<td>-0.0548864</td>
</tr>
<tr>
<td>55</td>
<td>-0.0324499</td>
<td>-0.0285602</td>
</tr>
<tr>
<td>50</td>
<td>-0.0183954</td>
<td>-0.0151124</td>
</tr>
<tr>
<td>45</td>
<td>-0.0097195</td>
<td>-0.0107919</td>
</tr>
<tr>
<td>40</td>
<td>-0.0064019</td>
<td>-0.0058037</td>
</tr>
</tbody>
</table>

- $X^2=3.397$ and p-value=0.639
- There is enough evidence to support the linear relationship
Parameter estimation

- All parameters of the model are considered to be fixed except for errors
- Maximum likelihood method is used for parameter estimation
- Simplified likelihood: \( L(\beta_0, \beta_1, \beta_2, \lambda, \sigma^2_\varepsilon | \mathbf{Y}) \)
- The standard errors of the estimates are computed based on the inverse of the Hessian matrix
- Delta method is used to obtain the standard error of stability estimates
- PROC MLMIXED of SAS® 9.1 (SAS Institute Inc., Cary, NC) is used for calculations
Estimates at storage temperature

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>1.04</td>
<td>0.019</td>
<td>1.00</td>
<td>1.07</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>-0.00001</td>
<td>0.00000048</td>
<td>-0.00002</td>
<td>-0.000004</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>-0.000003</td>
<td>0.0000003</td>
<td>-0.000004</td>
<td>-0.000003</td>
</tr>
<tr>
<td>Lag phase</td>
<td>164</td>
<td>15.73</td>
<td>133</td>
<td>195</td>
</tr>
<tr>
<td>Shelf life</td>
<td>356</td>
<td>15.09</td>
<td>326</td>
<td>386</td>
</tr>
</tbody>
</table>
Degradation at storage temperature

Lag Phase
Degradation Phase
Performance outside specifications
Arrhenius model is not appropriate

Conditions

- A zero- or first-order kinetics reaction takes place at each elevated temperature as well as storage temperature
- Linear relationship between degradation rate (LOG transformation) and temperature (inverse)
- The same model is used to fit the degradation patterns at each temperature
- Analytical accuracy should not be compromised during the course of the study in order to distinguish between the degradation rates at each temperature
Arrhenius model is not appropriate

Shelf-life using product similarity

- Similar product (family) to be used as control. The same family, the same kinetics of the degradation
- Comparisons to a product with a known stability
- Side by side testing of the control and test product at different elevated temperatures
- Experimental protocols are similar to the protocols when the Arrhenius equation is used
- Degradation patterns of a family of products at different elevated temperatures are modeled
- Prediction of shelf-life is based on product similarities rather than the relationship between degradation rate and temperature
Arrhenius model is not appropriate

- **Product**
  - IMMUNO-TROL™ Low Cells reagent is a single level, assayed, whole blood quality control product that provides a positive cell control at lower level of CD4 cells
  - Recommended storage temperature is 2°C to 8°C

- **Accelerated test**
  - Three elevated temperatures, 50°C, 45°C, 37°C
  - Three lots
  - MFI of CD3+ CYTO-STAT® tetraCHROME™ CD45-FITC / CD4-RD1 / CD8-ECD / CD3-PC5 is recorded
Arrhenius model is not appropriate

Control product

- IMMUNO-TROL™ Cells reagent
- IMMUNO-TROL™ Cells reagent has a stability of 9 months
- IMMUNO-TROL™ Cells and IMMUNO-TROL™ Low Cells reagent are side by side tested at three elevated temperatures, 50°C, 45°C, and 37°C
Arrhenius model is not appropriate

Degradation model

- Degradation model

\[ Y = \alpha [1 + \beta_0 \exp(-\beta_1 t)] + \varepsilon \]

\( t \) is time, \( \beta_1 \) is the degradation rate, \( \alpha (1 + \beta_0) \) is the expected MFI at time zero, \( \alpha \) is the minimum MFI after all the degradation has occurred

- Critical point of failure

\[ Y_{\text{Crit}} = \alpha (1 + \beta_0)/2 \]

- Predicted stability

\[ t_{\text{Stab}} = \frac{\log(Y_{\text{Crit}} - \alpha) - \log(\alpha) - \log(\beta_0)}{-\beta_1} \]
Arrhenius model is not appropriate
Comparison of degradations
Arrhenius model is not appropriate
Comparison of stability

<table>
<thead>
<tr>
<th>Temperature (C°)</th>
<th>Parameter</th>
<th>Control Lots</th>
<th></th>
<th>Test Lots</th>
<th></th>
<th>Comparison (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Estimate</td>
<td>Lower</td>
<td>Upper</td>
<td>Estimate</td>
<td>Lower</td>
</tr>
<tr>
<td>37</td>
<td>Deg. rate</td>
<td>0.001</td>
<td>0</td>
<td>0.001</td>
<td>0.001</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stability</td>
<td>1614</td>
<td>463</td>
<td>2764</td>
<td>1474</td>
<td>709</td>
</tr>
<tr>
<td>45</td>
<td>Deg. rate</td>
<td>0.004</td>
<td>0.001</td>
<td>0.006</td>
<td>0.003</td>
<td>0.002</td>
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<tr>
<td></td>
<td>Stability</td>
<td>269</td>
<td>165</td>
<td>373</td>
<td>281</td>
<td>204</td>
</tr>
<tr>
<td>50</td>
<td>Deg. rate</td>
<td>0.052</td>
<td>0.007</td>
<td>0.096</td>
<td>0.058</td>
<td>0.006</td>
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<tr>
<td></td>
<td>Stability</td>
<td>19</td>
<td>9</td>
<td>29</td>
<td>16</td>
<td>10</td>
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</tbody>
</table>
Arrhenius model is not appropriate

Comparison

<table>
<thead>
<tr>
<th>MFI</th>
<th>Control Lots</th>
<th>Test Lots</th>
<th>Comparison (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°C</td>
<td>45°C</td>
<td>50°C</td>
</tr>
<tr>
<td>Initial</td>
<td>23.6</td>
<td>24.1</td>
<td>24.9</td>
</tr>
<tr>
<td>Minimum</td>
<td>4.4</td>
<td>4.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Time</td>
<td>14436</td>
<td>2936</td>
<td>168</td>
</tr>
</tbody>
</table>
Questions, comments, suggestions.....