Improvement of Pipetting for a Pooling System:

Pooling Wizard software[3] operating the Microlab® STAR IVD[4] and Microlab® STAR*let* IVD[4] pipettor as pooling instruments

[3] *Pooling Wizard* is a trademark of Apartis Information Management
[4] Microlab® STAR IVD and/or Microlab® STARlet IVD are registered trademarks of Hamilton Corporation

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Background

Project was to analyze whether pipetting quality can be improved on a pipetting system for pooling, which has pressure measurement implemented (i.e. TADM® - Total Aspiration Dispense Monitoring for the Hamilton Microlab® STAR IVD and Microlab® STAR*let* IVD).

Used pipetting system already contains pressure control range definitions (Figure 1) in order to detect problematic pipetting events (Figure 2) and mark those pipettings as invalid.

Pressure control requires defining "guard bands", which set the acceptable range within a lower limit curve and a higher limit curve.

Figure 1: Guard Band Overview (Good Pipetting)

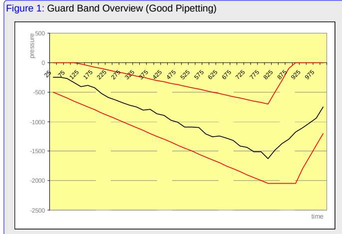
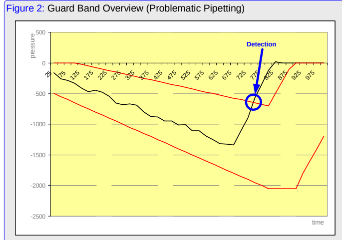


Figure 2: Guard Band Overview (Problematic Pipetting)



Design

The quality of pressure control depends on the width of the guard bands. Too broad of guard bands may not identify problematic pipettings (Figure 3).

Theoretically a narrow guard band provides higher quality. However, in practice a guard band that is too narrow may invalidate acceptable pipetting events (Figure 4).

The goal has been to identify the optimal settings for each pair of guard bands. The complexity has been given by the number of guard bands needed, i.e. one setting for each different volume, container, aspirate/dispense and tip status (new vs. wet).

Figure 3: Broad Guard Band with undetected pipetting error (e.g. Blot Clot)

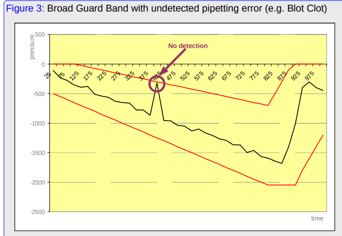
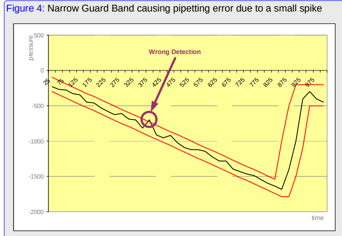


Figure 4: Narrow Guard Band causing pipetting error due to a small spike



The *Pooling Wizard* currently supports 5 different primary pooling methods and 13 different secondary pooling methods for resolution testing (Table 1).

Secondary pool volume is 1ml. Overall this required optimizing 47 different guard bands (Table 2) including the different pressure values for new tips versus wet tips (subsequent pipetting for one primary tube).

Table 1: List of Pooling Options

PRIMARY POOLING					
Pool size	96	48	24	6	1
	with or without Deep Well Plate				

SECONDARY POOLING					
Repeat	96	48	24	6	1
	with or without Deep Well Plate				
2D Pooling	96	48	24	--	--
	with or without Deep Well Plate				
Confirmation	96	48	24	--	--
	with or without Deep Well Plate				
Resolution	--	--	--	--	1
	with or without Deep Well Plate				
Repeat (+) [1)]	--	--	--	--	1
	with or without Deep Well Plate				

[1)] Repeat (+) = Repeat positive (reactive) test in duplicate

Table 2: List of Pipetting Configurations

Container		PBT [1)]		SPT [2)]		DEEP WELL PLATE				POOLING PLATE			
Mode		Aspirate		Dispense		Aspirate		Dispense		Aspirate		Dispense	
Tip		New	Wet	New	Wet	New	Wet	New	Wet	New	Wet	New	Wet
Volume	1000 µl	X	X	X	X	X	X	X	X	--	--	--	--
	700µl	--	X	--	--	--	--	X	X	--	--	--	--
	500µl	X	--	X	X	X	X	--	--	X	X	--	--
	334µl	X	--	--	--	--	--	--	--	--	--	--	--
	250µl	X	--	X	X	X	X	--	--	X	X	--	--
	167µl	X	--	X	X	X	X	--	--	--	--	--	--
	135µl	X	--	--	--	--	X	--	--	--	--	X	X
	125µl	X	--	X	X	X	X	--	--	X	X	--	--
	92µl	X	--	X	X	X	X	--	--	--	--	--	--

[1)] PBT = Primary Blood Tube, [2)] SPT = Secondary Pool Tube

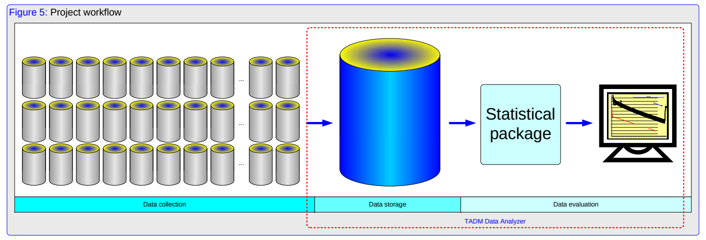
Results

The scope of this project was to evaluate whether pipetting guard bands can be optimized using statistical methods. Therefore multiple pipetting test runs for different scenarios were performed. One possible option was to create a test method for each scenario. The second option was to use the *Pooling Wizard*, which creates all pipetting methods including all single steps on demand. The second option has been chosen by the development team, since this option guarantees 100% similarity of test methods and final methods and so provides higher quality to the end product.

Data was collected in single data bases, one for each pooling run. Each data base contains all curves for a pooling run, i.e. one curve for each step. Data collection happened on different instruments in parallel and at different sites.

One part of the project was to setup a master database, where all single data bases could be uploaded. Statistical evaluation happened afterwards. Apartis developed a **TADM data analyzer** tool, which consists of a central Oracle™ database, a decentral upload tool and a statistical package (Figure 5).

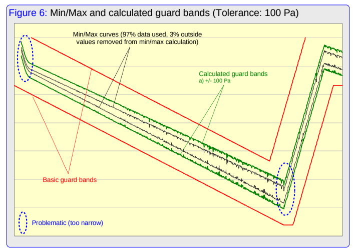
Figure 5: Project workflow



The statistical package has been optimized for curve evaluation and contains various statistical functions for curves: Average curves, minimum curves, maximum curves, moving average curves, standard deviation curves (1x, 2x, 3x) and derivation curves (1st, 2nd).

The master database has been built as a data warehouse with dimensions (e.g. site, instrument, channel, date/time, pooling run) and measures (e.g. pressure curves). The statistical package supports any combination of query parameters. This has been proved as valuable to compare potential differences between sites, instruments, channels, pooling runs and even time impact.

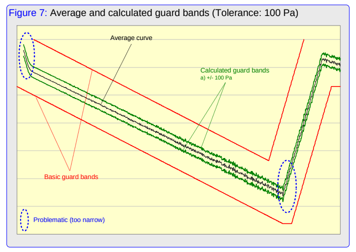
Figure 6: Min/Max and calculated guard bands (Tolerance: 100 Pa)



One sample guard band is used to explain the findings in this publication.

The first iteration uses all measured curves. A reduction of 3% data is performed for each measure point in order to remove spikes. Afterwards Min/Max curves and guard bands with a tolerance of 100 Pa [Pascal] are calculated (Figure 6).

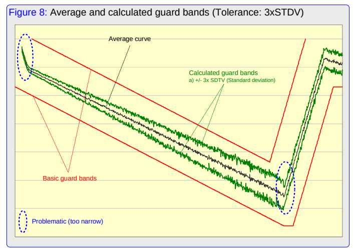
Figure 7: Average and calculated guard bands (Tolerance: 100 Pa)



Second iteration calculates the average curve for measure points and uses a tolerance band of 100 Pa again (Figure 7).

Since both iterations show too narrow guard bands during start and end of pipettings, standard deviation (here: 3x STDV) is introduced for third iteration (Figure 8).

Figure 8: Average and calculated guard bands (Tolerance: 3xSTDV)



Fourth iteration then combines third and second iteration - 3x STDV plus 100 Pa tolerance band (Figure 9). The remaining problem stays with the calculated *noise* on those guard bands.

Therefore fifth iteration adds a smooth algorithm (Moving average) to calculate the optimized guard bands (Figure 10) for a statistical evaluation approach.

Figure 9: Average and calculated guard bands (Tolerance: 3xSTDV + 100 Pa)

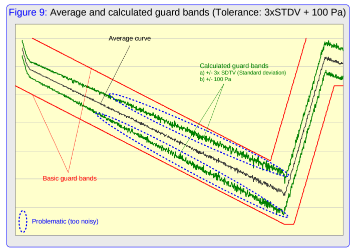
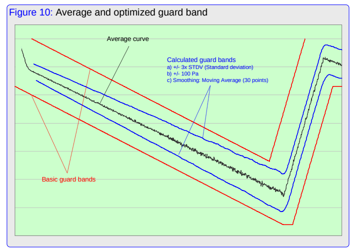


Figure 10: Average and optimized guard band



Summary

Using guard bands to monitor pipetting pressure is a reliable and well established method to improve pipetting quality. A central database that combines curves from multiple sites, instruments and runs is of essential help. The described statistical method allows for increasingly optimized guard band settings as more data is collected and evaluated.

Pipetting quality can be improved as a result.