

# Underfilled Blood Samples: An important source of error and improved detection using an automated Sample Volume Check on Sysmex CS analysers

Anita Woolley, Peter Brown, Steve Kitchen.

Coagulation Department, Royal Hallamshire Hospital, Sheffield, UK.

## Introduction

The pre-analytical phase is the source of the majority of errors in laboratory medicine.

Under-filling of citrated blood samples can affect the results of clotting tests because there is an alteration in the ratio of blood to anticoagulant.

The decision to accept a sample on the basis of sufficient fill has most often been done by visual scrutiny and therefore subject to vigilance and operator decision.

However automated Sample Volume Check is now available on the Sysmex CS series analysers - CS2100i and CS5100.

## Objectives

- To assess the impact of introducing automated volume detection on sample acceptance and rejection due to under-filling.
- To assess the effects of under-filling on the results of routine coagulation tests.

## Background

The Sysmex CS2100i/CS5100 analysers have a function called Sample Volume Check that can verify whether the correct volume of blood is collected into the sample tube.

The Sample Volume Check is a function that confirms the correct blood volume by checking the liquid level of the sample at the time when the sample is aspirated for analysis.

The volume check can be set up for various commonly used sample tube types

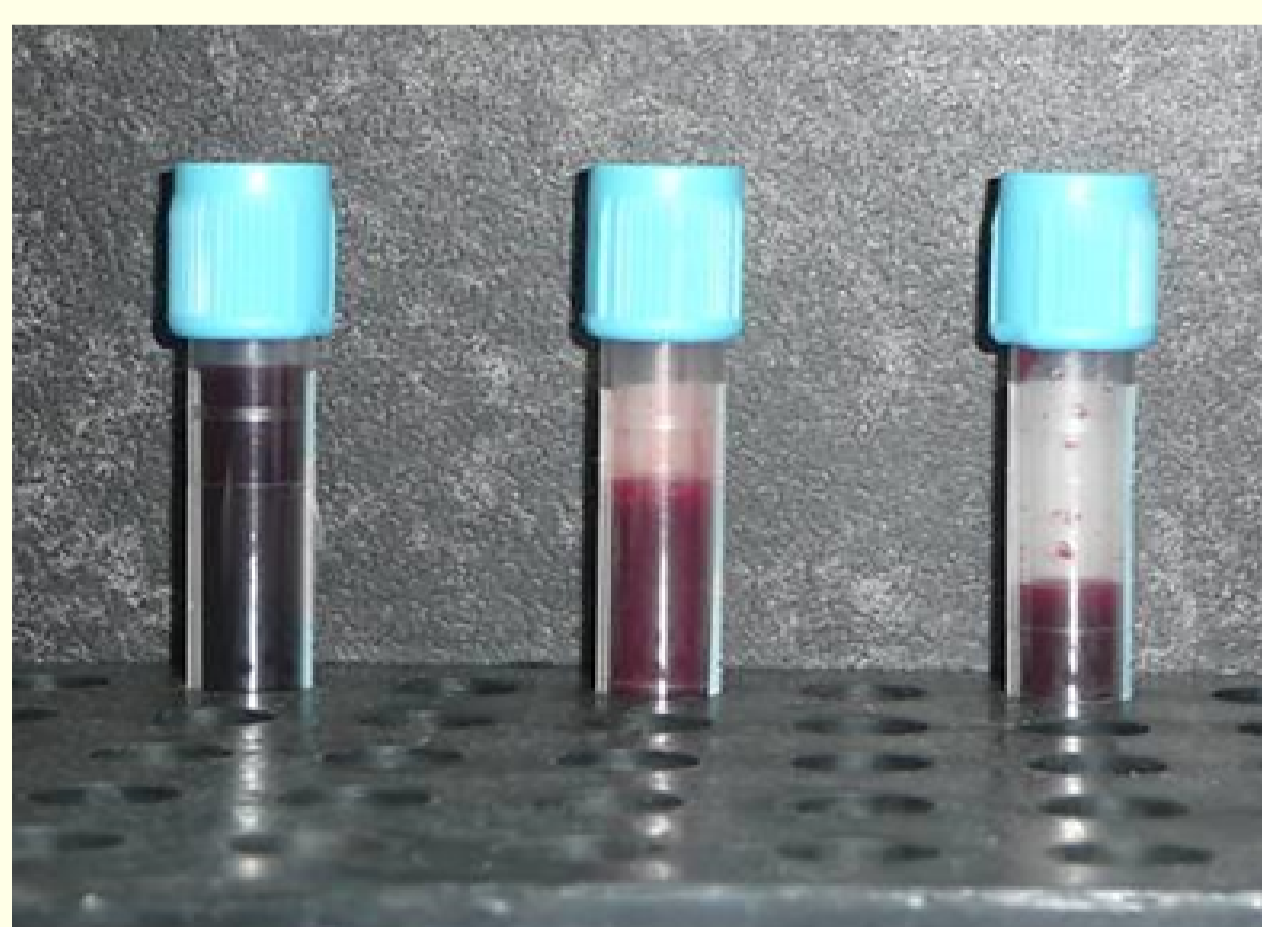
In blood samples collected for coagulation tests, the anticoagulant (sodium citrate) is mixed with blood at a ratio of 1:9

Sodium citrate reacts with calcium to produce calcium citrate which removes calcium from the blood causing the anticoagulant effect. When there is a high proportion of residual anticoagulant in the sample, the calcium concentration in subsequent tests is reduced often causing the coagulation time to become prolonged.

## Samples

- Citrate samples (3ml BD Vacutainer Plus) received within our routine service laboratory which were less than 80% of the target fill were retained.
- Samples from the same patients which contained 90-100% of the target fill and were received within 4 hours of the under-filled sample being taken were retained.
- All retained samples were separated and the plasmas stored at -80°C until tested
- These retained samples were analysed for the appropriate routine coagulation tests.
- All patients samples tested had a normal haematocrit.
- N = 45

## Vacutainer Plus BD 3ml fill tubes 100% 80% 60% of target fill



## Tests/Reagents

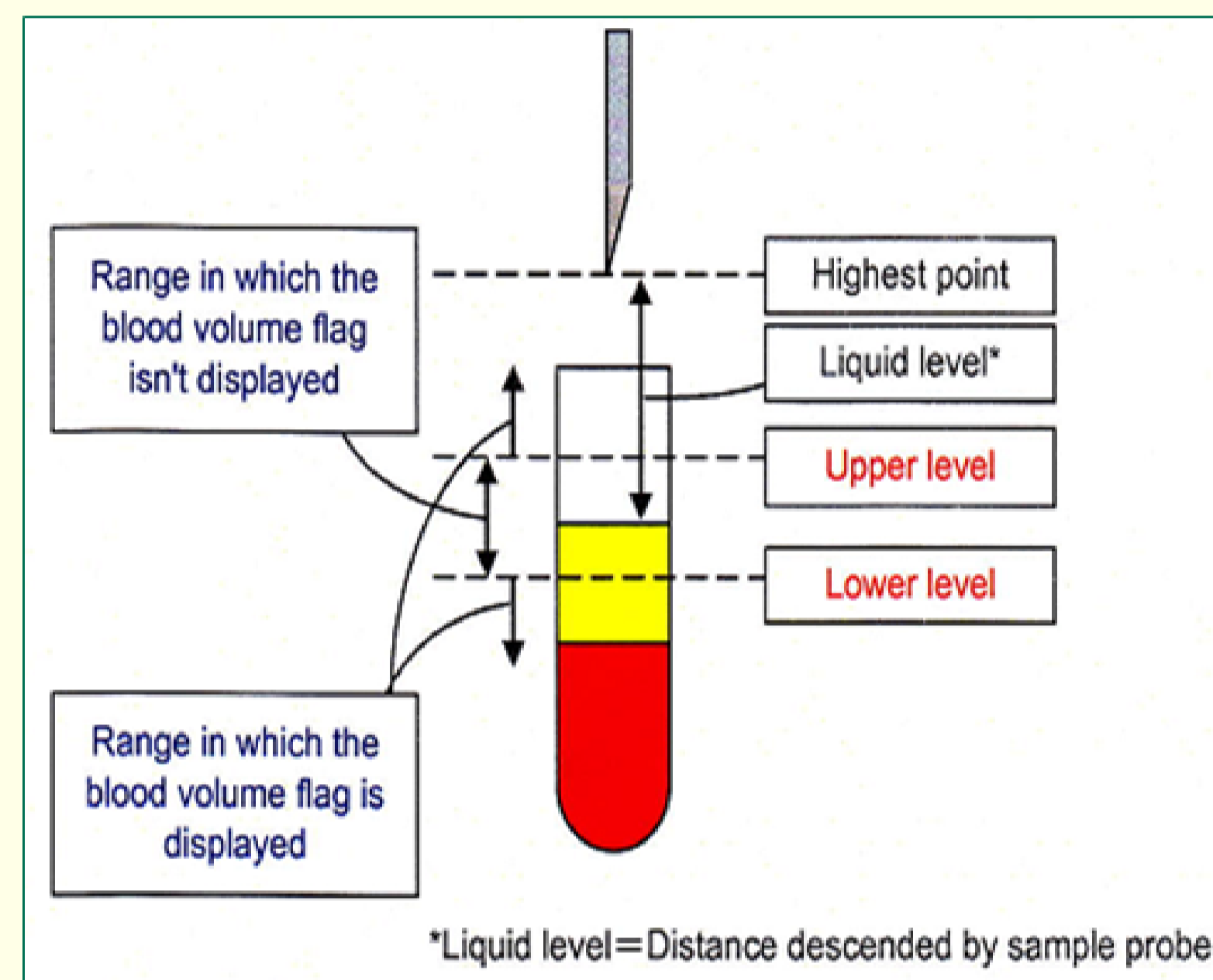
Prothrombin Time (PT)	Dade Innovin (Siemens)
Activated Partial Thromboplastin Time (APTT)	Dade Actin FS (Siemens)
Clauss Fibrinogen (CFbg)	Dade Thrombin (Siemens)
Thrombin Time (TT)	Dade Thromboclotin (Siemens)

## Methods

- The Sysmex CS2100i and CS5100 analysers were calibrated using the Sample Volume Check method issued by Sysmex.
- A previous study showed that samples in 3ml Vacutainer Plus citrate bottles should not be tested for routine coagulation assays if less than 80% full therefore we used this %fill in our calibration procedure.
- All samples that were rejected due to visual checking or were flagged by the analyser as underfilled were compared to the repeat adequately filled sample.
- An audit of rejected samples was performed for 2 weeks following the introduction of the analyser 'Sample Volume Check'.

## Sample Volume Check - Analysis Principle

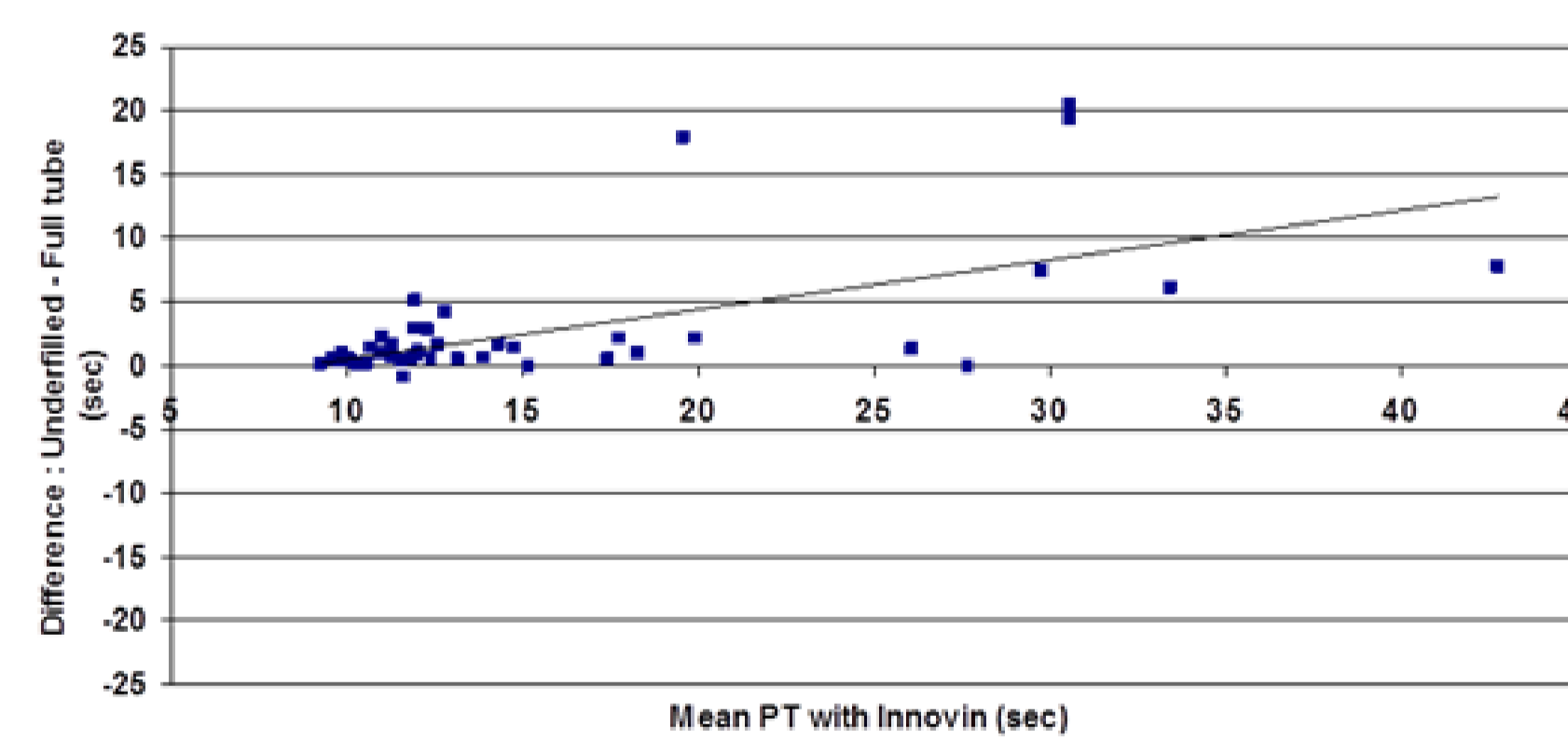
- This is a function on the Sysmex CS series analysers that checks whether the liquid level of the blood sample falls within a set range at the time when the sample is aspirated for analysis.
- When this level is outside the set range, a blood volume error flag is displayed on the analyser against the sample result



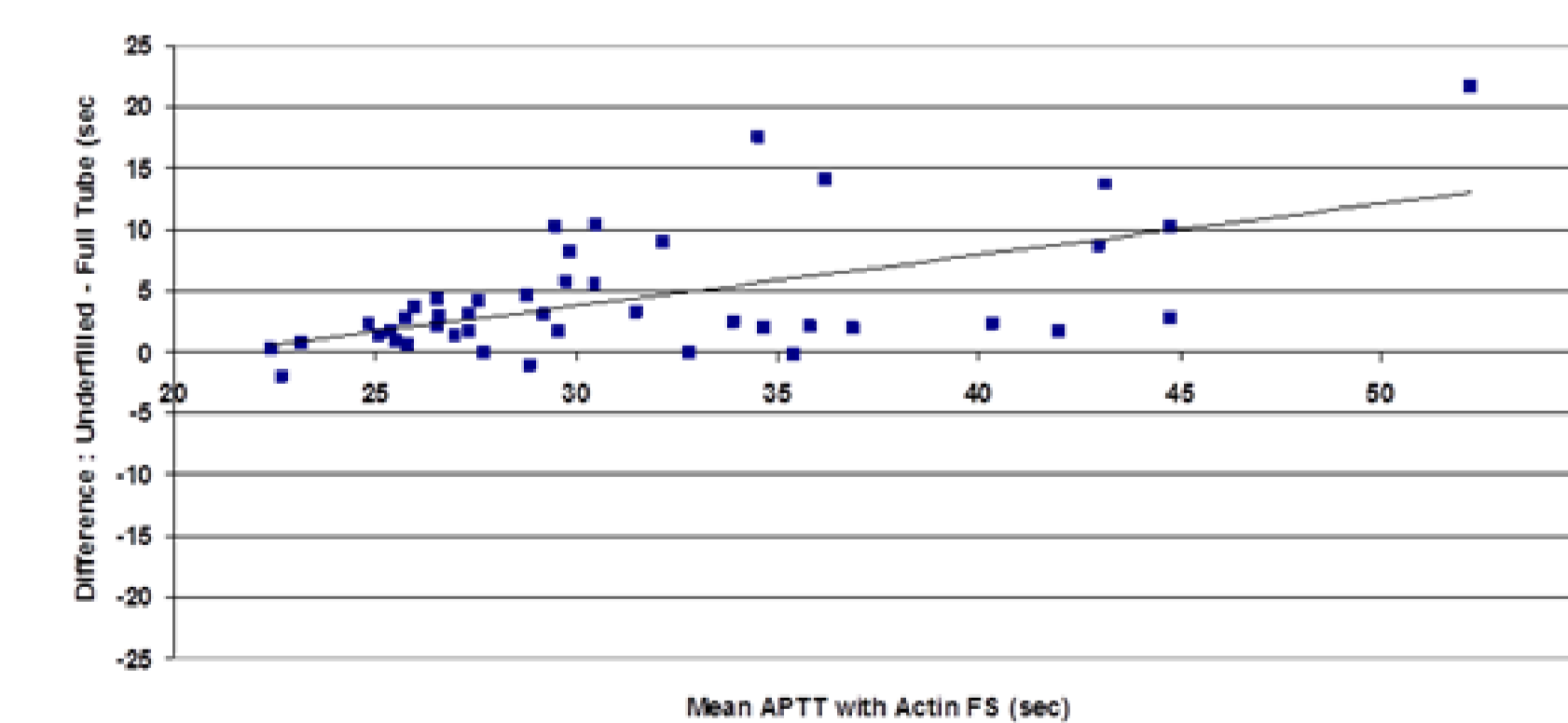
## Results

Mean results for full and underfilled sample tubes				
	PT (secs)	APTT (secs)	CFbg (g/L)	TT (secs)
Full	14.1	29.3	3.9	15.9
Underfilled	16.8	33.6	3.7	17.9
Max difference	20.4	21.7	1.6	5.8

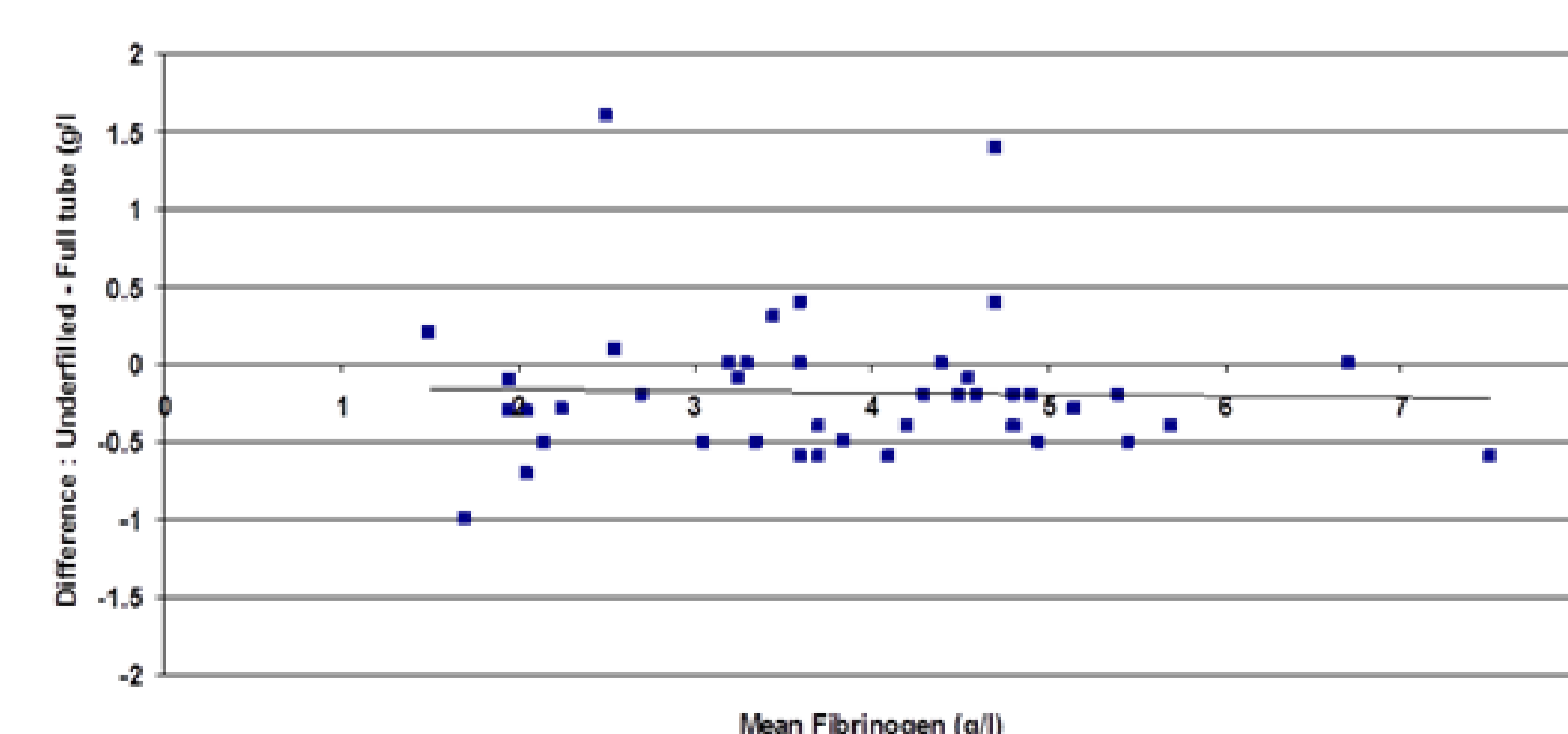
### Prothrombin Times in Underfilled Tubes



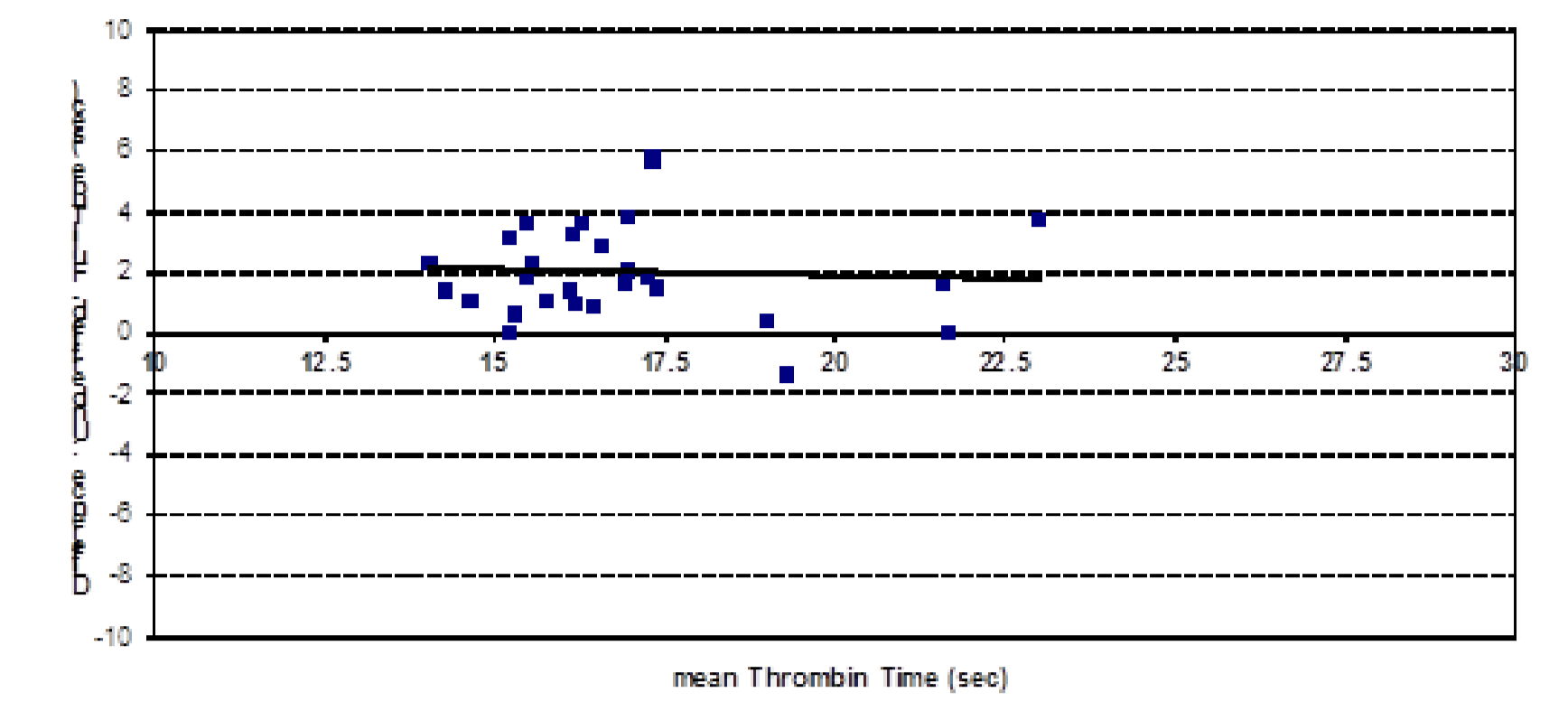
### APTTs in Underfilled Tubes



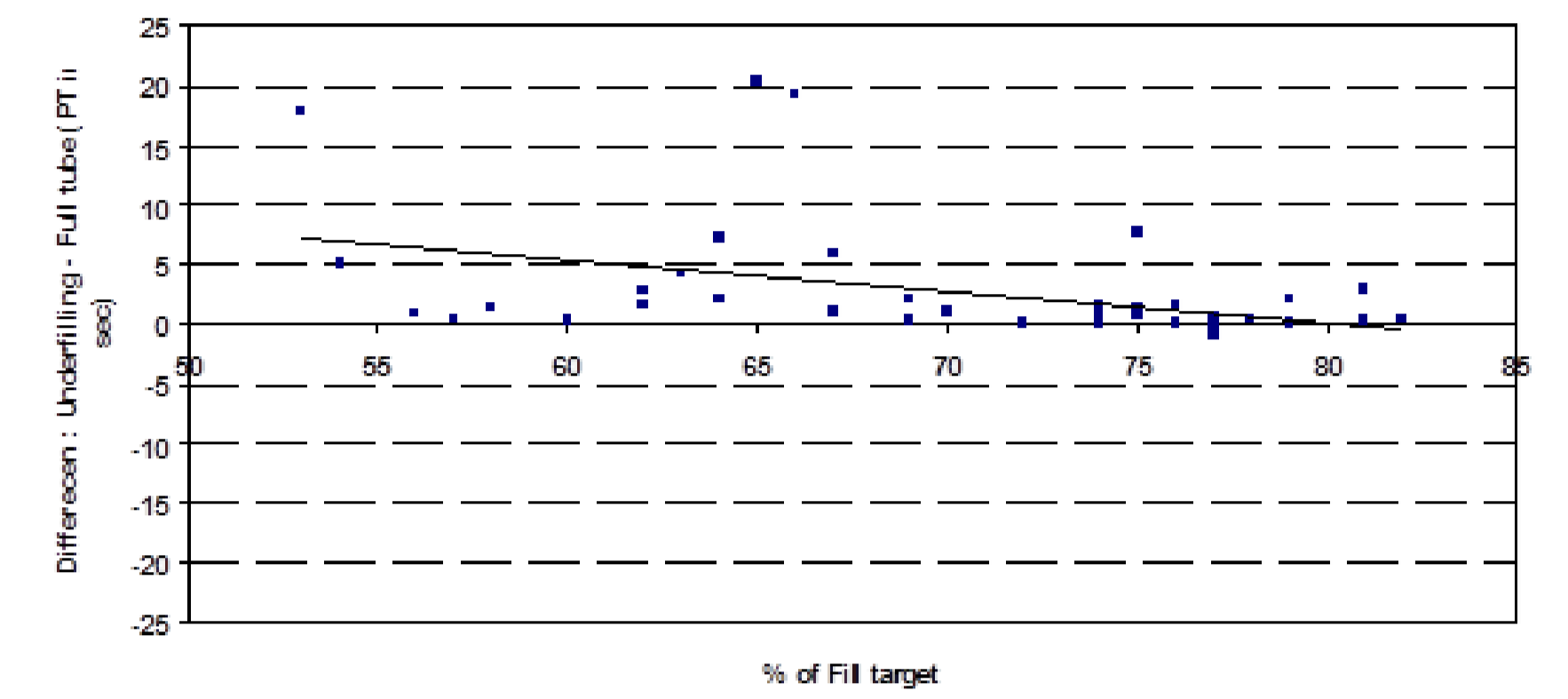
### Fibrinogen (Clauss assay) in Underfilled Tubes



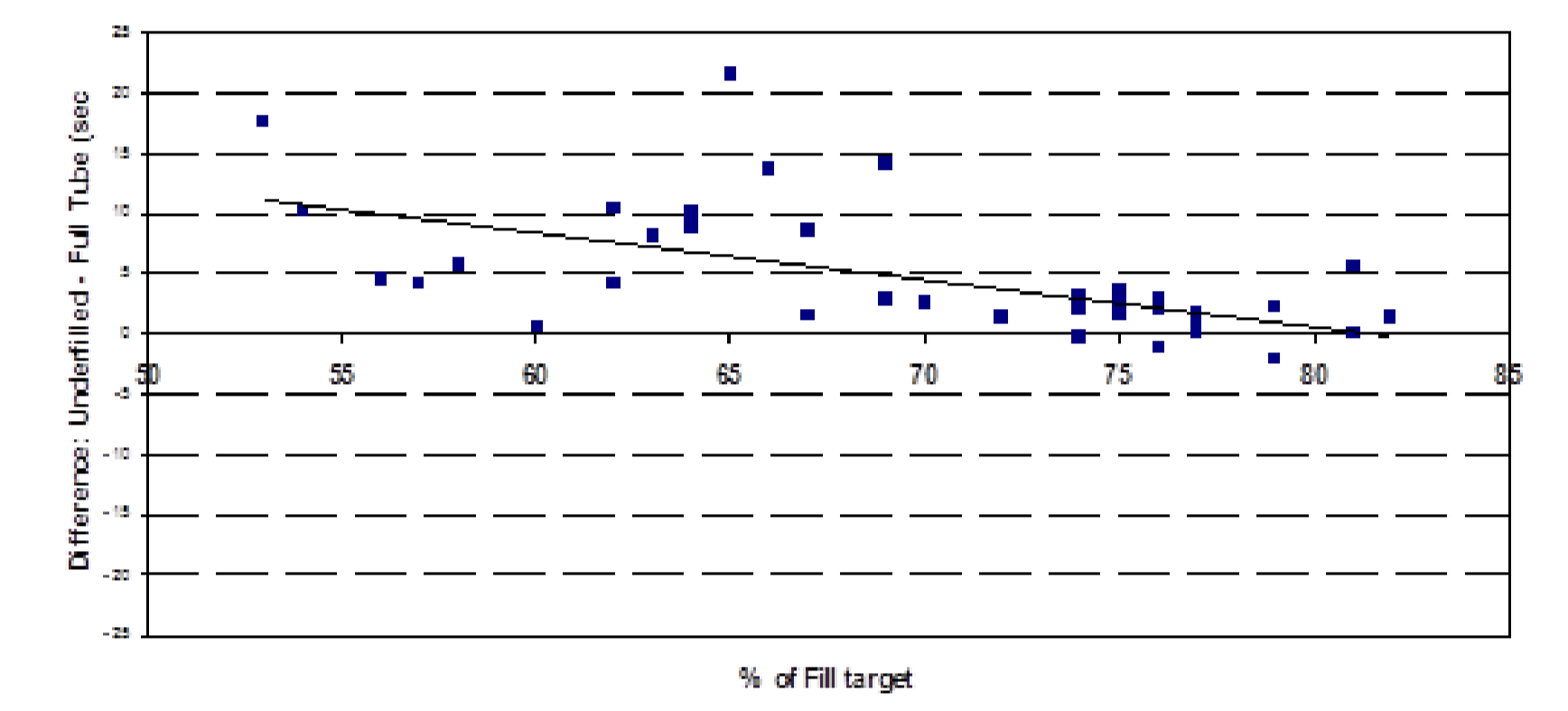
### Thrombin Times in Underfilled Samples



### Difference in PT in relation to degree of Underfilling



### Difference in APTT in relation to degree of Underfilling



### Audit Outcome

Pre 'Sample Volume Check' installation	
Total samples received	3507
Number underfilled samples rejected	20
% rejected	0.57
Equates to total rejections per year	520
Post 'Sample Volume Check' installation	
Total samples received	3838
Number underfilled samples rejected	33
% rejected	0.86
Equates to total rejections per year	858
Possible number of erroneous results per year	338

## Summary

- PT/APTT/TT were all significantly longer in underfilled samples (p,0.001).
- There was a significant negative correlation between the %fill of the samples and the differences in PT and APTT test results in full and underfilled tubes – thus the lower the %fill the greater was the prolongation of PT and APTT.
- Approximately 20% of underfilled samples had falsely elevated APTTs.
- Approximately 0.6% of 120,000 samples were detected as underfilled by visual scrutiny with a mean fill of 71% and a range of 53-79%
- After introduction of automated Sample Volume Check the detection rate of underfilled samples increased to almost 1%

## Conclusion

We conclude that blood samples (from subjects with a normal haematocrit) collected into 3ml BD Vacutainer Plus tubes should not be accepted for analysis of coagulation routine tests if less than 80% full.

We also noted that the automated Sample Volume Check on the Sysmex CS series analysers significantly improved the detection rate of unacceptable underfilled samples, thus preventing the issue of erroneous results.

## Acknowledgments

We thank Sysmex (UK) Ltd for permission to use the Sample Volume Check diagram and information and for support for this presentation.

