Compiled by	**Job Title**	**Date**	**Signature**
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A Contents

B Analysis of Results

The trials were carried out in March 2009.

B.1 Screw Caps

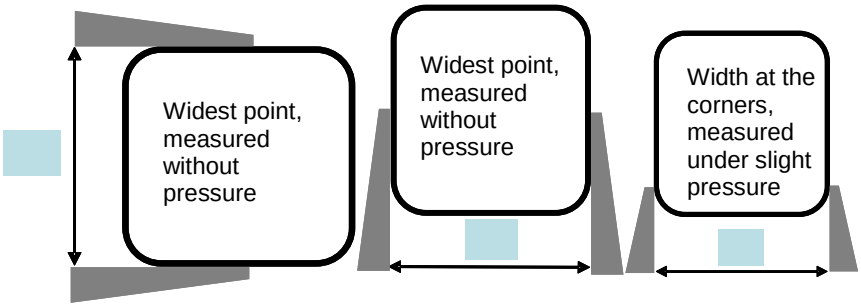
Visual check: No optically detectable defects.

	Diameter of lower ring [mm]	Height [mm]	Mass [g]
Average	**41.72**	**27.68**	**8.71**
Maximum	42.02	28.28	8.80
Minimum	41.28	27.51	8.53
Standard deviation	0.1637	0.1723	0.0531
Confidence interval 95% (±2 x standard deviation)	±0.14	±0.14	±0.083

No. of bottles: n = 35

B.2 The Bottle

Dimensions.



MEASUREMENTS	Height [mm]	Width (AF) [mm]	Length (BF) [mm]	Length (KF) [mm]	Mass [g]
Average	**168 (*)**	**76.25**	**76.19**	**76.27**	**72.79**
Maximum	n. a.	76.94	76.61	76.77	74.23
Minimum	n. a.	75.12	75.73	75.77	71.91
Min-Max difference	n. a.	1.82	0.88	1.00	2.32
Standard deviation	n. a.	0.296	0.2404	0.2955	0.6836
Confidence interval 95% (±2 x standard deviation)	n.a.	±0.068	±0.30	±0.078	±0.12

(*):n=5

No. of bottles: n = 35

Visual check: The bottle looks OK and complies (optically) with our expectations.

B.3 Performance Test - Manual closure of the bottle using 5 Nm of torque

No problems.
Visual check: Screw cap optically OK.
Visual check after 3 hours at room temperature: Also OK.

No. of bottles: n = 30

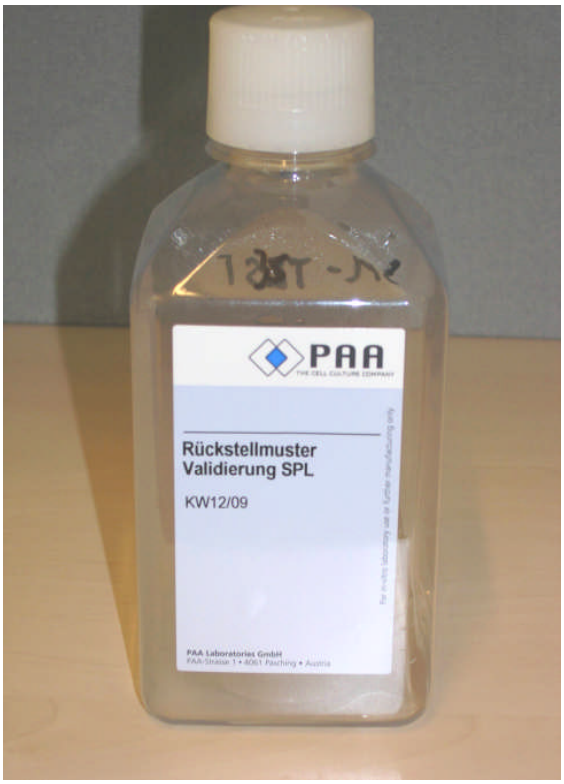


Figure 1

B.4 Performance Test - Filling the bottles

B.4.1 Fully automatic filling

1000 bottles were filled using the full automatic fill line.
20 bottles were labelled using the labelling machine.
The fill line required no modification.
The labelling machine needed to be fitted with a **new (slightly smaller) screw jack**.
The labelling worked perfectly well. All screw caps were still fully sound after two weeks' storage at room temperature.
3 Nm torque were required to open the bottles that were closed using 3 Nm.
5 Nm torque were required to open the bottles that were closed using 5 Nm.

B.4.2 Media-fill

All of the media-filled bottles were still sterile two weeks after filling.

B.5 Performance Test - 1st Freezing

B.5.1 Freezing at -80°C, storage at between -15°C and -25°C

Measured at the widest point after freezing	A_F [mm]	Before freezing [mm]
Average	**76.79**	**76.27**
Maximum	79.90	76.94
Minimum	75.4	75.12
Min-Max difference	4.5	1.82
Standard deviation	1.1165	0.2960
Confidence interval 95% (±2 x standard deviation)	±2.89	±0.30

No. of bottles: n = 30

Frozen in dry ice at -80 °C (n=30): 100% deformation
The bottles were slightly deformed after 2 hours at -80°C and then 14 hours at between -15°C and -25 °C, but still fulfil PAA's standards.

B.6 Performance Test - 1st Thaw

The bottles were thawed out at 37 °C in a water bath.

After thawing - Measured at the widest point	A_F [mm]	Before freezing [mm]
Average	**77.23**	**76.27**
Maximum	78.05	76.94
Minimum	76.42	75.12
Min-Max difference	1.63	1.82
Standard deviation	0.4799	0.2960
Confidence interval 95% (±2 x standard deviation)	±1.1	±0.30

No. of bottles: n = 30

Visual check: The bottles begin to regain their original form. After thawing, the bottles still fulfil PAA's standards.

B.7 Performance Test - 1st Heat Inactivation

Heat Inactivation:

1. Thaw at 37 °C
2. Heating phase: 09.05 - 09.25 RT ==> 56°C 20min
3. Incubation phase: 09.25 - 09.55 56-58°C 30min

After Heat Inactivation - Measured at the widest point	A_F [mm]	Before freezing [mm]
Average	**80.78**	**76.27**
Maximum	82.16	76.94
Minimum	78.9	75.12
Min-Max difference	3.26	1.82
Standard deviation	0.9973	0.2960
Confidence interval 95% (±2 x standard deviation)	±1.6	±0.30

No. of bottles: n = 30

Visual check: The bottles fulfil PAA's standards.

Figure 2: Bottle after Heat Inactivation (HI)

B.8 Performance Test - 2nd Freezing (with serum)

Instead of being frozen in dry ice (as in the 1st freezing trials) the bottles were here frozen for up to 12 hours at between -15°C and -30°C using a shock freezer.

200 x 650ml bottles were filled with FBS. 50 of these were heat inactivated.
The bottles were then frozen as follows:

75 FBS bottles without heat inactivation using a shock freezer (-30°C)
25 heat inactivated FBS bottles using a shock freezer (-30°C)

75 FBS bottles without heat inactivation at -15°C
25 heat inactivated FBS bottles at -15°C

The frozen bottles were then packed into the usual Styrofoam boxes.

Heat inactivated FBS at <-15°C

Figure 3

Heat inactivated FBS at -30°C

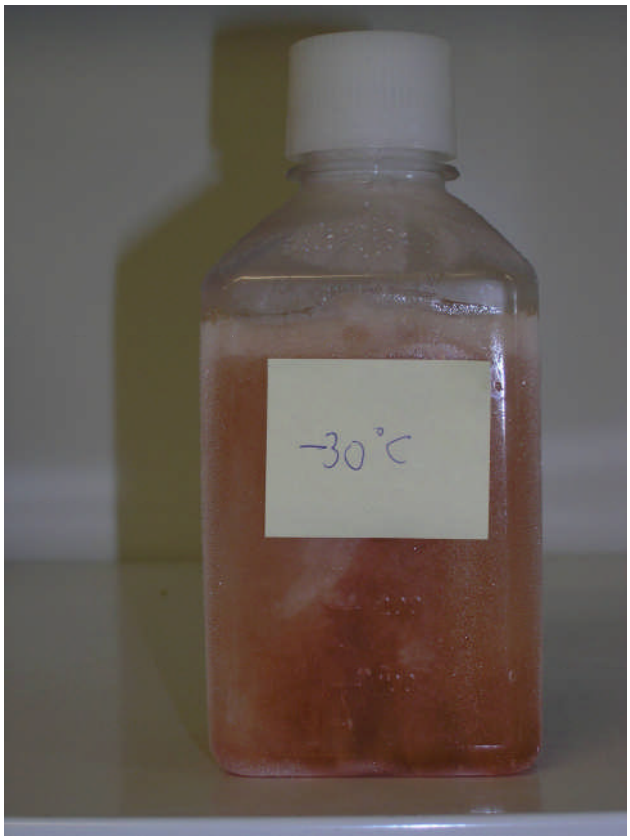


Figure 4

FBS bottles without Heat Inactivation at <-15°C
20 bottles per box

Figure 5

FBS bottles without heat inactivation <-30°C
20 bottles per box

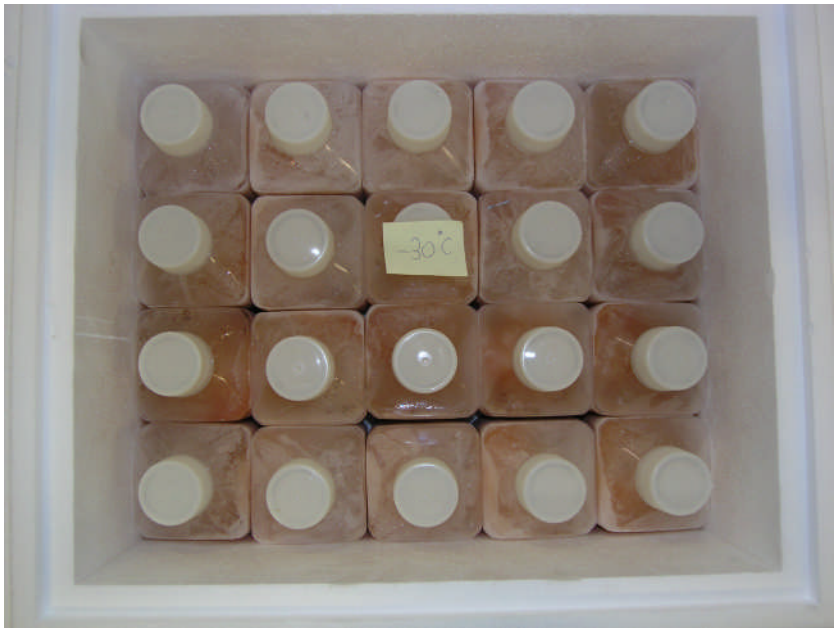


Figure 6

Heat inactivated FBS bottles at <-15°C
16 bottles per box

Figure 7

Heat inactivated FBS bottles at -30°C
17 bottles per box

Figure 8

Conclusion:
The bottles do not distend significantly when filled with FBS. At the end of the above described tests the bottles did not show any obvious defects or flaws.

B.9 Performance Test - 2nd Thaw

The bottles were thawed out at 37 °C in a water bath.

After 2nd thaw - Measured at the widest point	A_F [mm]	Before thawing [mm]
Average	**82.17**	**76.27**
Maximum	83.33	76.94
Minimum	79.5	75.12
Min-Max difference	3.83	1.82
Standard deviation	0.8626	0.2960
Confidence interval 95% (±2 x standard deviation)	±1.4	±0.30

No. of bottles: n = 30

Visual check: Bottles are optically OK.

C Summary

Result:

Optically, all bottles looked OK following the tests.

Other observations:

- The bottles are fully suitable for being used as media and serum bottles
- The bottles performed perfectly well during the full automatic fill process
- The graduation fully complied with the actually measured content
- The shrink sealing of the trays was slightly too tight
- Storage tests for 1000 bottles revealed no defect closures – no screw caps were torn