CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

102301

The AOAC Research Institute hereby certifies the method known as:

Oculer Rapid 930 CS-Check™ Commercial Sterility Testing Vials

manufactured by

Oculer Ltd
Unit 2 Shannonside Business Park
Birdhill, Co. Tipperary
Ireland

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

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METHOD NAME
Oculer Rapid 930 CS-Check™ Commercial Sterility Testing Vials (50 vials)

CATALOG NUMBER
OC-CSC-050

INDEPENDENT LABORATORY
Campden BRI
Station Road
Chipping Campden
Gloucestershire, GL55 6LC
United Kingdom

APPLICABILITY OF METHOD
Target organism – Aerobic mesophilic microorganisms.

Matrixes – Ultra-high temperature (UHT) milk.

Performance claims – The study data were unable to detect a statistical difference in results between the Oculer Rapid 930 CS-Check™ method (hold for 5 days at 30°C) and the culture-based reference method indicated by European Directive 92/46 Annex C Chapter 1.A.4.b (hold for 15 days at 30°C) (2) for UHT milk.

ORIGINAL CERTIFICATION DATE **October 30, 2023**	**CERTIFICATION RENEWAL RECORD** **New approval 2023.**
METHOD MODIFICATION RECORD **NONE**	**SUMMARY OF MODIFICATION** **NONE**
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PRINCIPLE OF THE METHOD (1)
The Oculer Rapid 930 CS-Check™ system uses vials which contain a broth able to support the growth of aerobic, mesophilic microorganisms (bacteria, yeasts and molds) present in heat processed liquid products. As growth of the organisms occurs, oxygen is used from within the media vial and this depletion is automatically detected using a platinum porphyrin sensor. The sensor is excited by incident light and the lifetime of the emitted light is measured by the instrument. The lifetime of the emitted light from each individual vial is measured in real time by the Oculer Rapid 930 Series instrument. The time to growth detection in the Oculer Rapid 930 CS-Check correlates to the level of microorganisms present in the sample, with higher levels of contamination having a shorter detection time.

DISCUSSION OF THE VALIDATION STUDY (1)
Examination of the performance of different batches of Oculer Rapid 930 CS-Check, the results showed that the vials were consistently produced and remain stable over a year of storage at room temperature (18-25°C). Furthermore, small variations in sample volume, changes in sample temperature (2-8°C or 18-25°C) and different mixing techniques had no impact on test performance. Performance of the test on three separate instruments showed consistent performance.
A panel of 51 (aerobic and facultative) reference cultures were selected for the inclusivity study, based on their link to hygiene and spoilage and 50% of these cultures were isolated from food.
The reference method used in the matrix study was the European Directive 92/46 Annex C, Chapter 1.A.4.b for UHT milk using ISO 4833-1:2013 for the plate count at 30°C. The methodology included 15 days of preincubation at 30°C and plating the samples on mPCA for colony counts after 72 h of incubation for the reference method. For the Oculer (candidate method) pre-incubation was for five days followed by up to 48 h incubation on the Oculer instrument. Using dPOD analysis, no statistical differences were detected between the Oculer Rapid 930 CS-Check methods and the reference method. These results emphasize the importance of the Oculer Rapid 930 CS-Check as a rapid method for commercial sterility tests. Use of the Oculer system results in a minimum of nine days time saving when compared to the reference method. This is largely achieved by the much greater volume of sample (after pre-incubation) analyzed when the Oculer system is used as compared to the reference method.

Table 4. Inclusivity List (1)

No.	Genus	Species	CRA[b] Number	Origin	Level inoculated (cfu/carton)	Oculer Rapid 930 CS-Check Time to Threshold	Oculer Rapid 930 CS-Check Result
1	*Raoultella*	*terrigena*	17343	Raw milk	9.6	00:00	+
2	*Enterobacter*	*cloacae*	1472	Dried milk	1.9	00:00	+
3	*Klebsiella*	*oxytoca*	8387	Water	2.1	00:00	+
4	*Kluyvera*	*ascorbata*	17126	Industrial	2.2	00:08	+
5	*Escherichia*	*adecarboxylata*	5501	Skim milk powder	10	00:00	+
6	*Klebsiella*	*trevisanii*	NCIMB[c] 8606	Ropy cream	2.4	27:01:00	+
7	*Pantoea*	*agglomerans*	17030	Pasteurized milk	1.8	00:08	+
8	*Aeromonas*	*hydrophila*	8388	Tin of milk with a fishy odor	2.5	03:50	+
9	*Escherichia*	*coli*	1476	Dried milk	1.6	00:12	+
10	*Rahnella*	*aqualtilis*	16911	Drinking water	1.4	00:00	+
11	*Bacillus*	*coagulans*	16586	Sterilized milk	3.4	08:22	+
12	*Bacillus*	*subtilis*	16579	Environmental	8.3	27:06:00	+
13	*Bacillus*	*weihenstephanensis*	16578	Pasteurized milk	9	03:57	+
14	*Bacillus*	*polymyxa*	7747	Unknown	2.8	00:31	+
15	*Bacillus*	*cereus*	7746	Milk or cream	8.5	10:59	+
16	*Bacillus*	*pseudomycoides*	16382	Soil in Sweden	4.6	29:26:00	+
17	*Bacillus*	*pumilus*	655	Chilled chicken in white wine sauce	4.1	02:16	+
18	*Lysinibacillus*	*sphaericus*	7746	Unknown	2.3	01:17	+
19	*Bacillus*	*thuringiensis*	NCIMB 9134	Flour moth	10	00:17	+
20	*Bacillus*	*licheniformis*	6335	Pesto sauce	8	07:31	+
21	*Paenibacillus*	*macerans*	16488	Unknown	3.8	06:25	+
22	*Brevibacillus*	*brevis*	7748	Unknown	3.8	03:09	+
23	*Aneurinibacillus*	*aneurinolyticus*	7751	Unknown	2.2	04:09	+
24	*Paenibacillus*	*pabuli*	16606	Barley	2.8	04:02	+
25	*Brevibacillus*	*aigri*	7749	Unknown	2.7	01:52	+

Table 4. Inclusivity List. Continued (1)

No.	Genus	Species	CRA[b] Number	Origin	Level inoculated (cfu/carton)	Oculer Rapid 930 CS-Check Time to Threshold	Oculer Rapid 930 CS-Check Result
26	*Staphylococcus*	*carnosus*	1123	Goat's milk	2.2	04:47	+
27	*Listeria*	*ivanovii*	16045	Soft cheese	1.8	01:42	+
28	*Streptococcus*	*thermophilus*	7675	Pasteurized milk	1	3:34:00	+
29	*Lactobacillus*	*acidophilus*	3910	Dairy product	1.8	00:04	+
30	*Carnobacterium*	*divergens*	8999	Brie	2.4	00:44	+
31	*Staphylococcus*	*saprophyticus*	3503	Distilled water environmental	8	02:48	+
32	*Micrococcus*	*luteus*	1513	Air sample	8.7	03:36	+
33	*Enterococcus*	*faecalis*	272	Dried milk powder	8.4	1:45:00	+
34	*Staphylococcus*	*cohnii*	409/3026	Skin	2.8	01:42	+
35	*Staphylococcus*	*aureus*	314	Slow cheese	1.5	00:12	+
36	*Staphylococcus*	*epidermis*	16030	Runway and can seam	9.7	0:04:00	+
37	*Pediococcus*	*pentosaceus*	100	Brine	3.4	05:53	+
38	*Listeria*	*monocytogenes 1/2a*	1100	Stilton	1.8	03:45	+
39	*Listeria*	*innocua*	16828	Cheese factory	8.5	3:06:00	+
40	*Staphylococcus*	*hominis*	16029	Unknown	9.3	02:15	+
41	*Lactococcus*	*lactis*	16659	Green ham	2.2	00:00	+
42	*Micrococcus*	*roseus*	7775	Water	1.2	00:00	+
43	*Streptococcus*	*lactis*	1511	Dried milk powder	2	00:00	+
44	*Enterococcus*	*malodoratus*	16860	Gouda cheese	8.6	00:00	+
45	*Enterococcus*	*pseudoavium*	16852	Cow udder - bovine mastitis	9.6	00:00	+
46	*Aureobasidium*	*pullulans*	16148	Soft drinks factory	3.2	36:05:00	+
47	*Byssochlamys*	*fulva*	16668	Pasteurized fruit juice	3.2	14:15	+
48	*Candida*	*krussei*	629	Yogurt base	4.2	03:12	+
49	*Kluyveromyces*	*marxianus*	6749	Dairy isolate	4.3	05:09	+
50	*Torulaspora*	*delbruekeii*	16154	Spoiled yogurt	9	1:16:00	+
51	*Aneurinibacillus*	*aneurinolyticus*	7758	human	10	00:27	+

[a]Result: "+" = strain detected, "-" = strain not detected. [b]Campden BRI Culture Collection, Chipping Campden, UK. [c]National Collection of Industrial, Food, and Marine Bacteria, Aberdeen, UK.

Table 5. Matrix study: Oculer Rapid 930 CS-Check presumptive results vs. confirmed results (1)

Matrix	Strain	Contamination level (cfu/g)[a]	N[b]	CS-Check presumptive results			CS-Check confirmed results				
				x[c]	POD_{CP}[d]	95% CI[f]	x	POD_{CC}[e]	95% CI[f]	$dPOD_{CP}$[g]	95% CI
UHT whole milk	*Bacillus subtilis* (CRA[h] 16597)	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0	-0.47, 0.47
		0.8	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.11, 0.21
		4.1	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0	-0.47, 0.47

[a] = Inoculum level per g of sample matrix.
[b]= Number of test potions.
[c]x = Number of positive test portions.
[d]POD_{CP} = Candidate method presumptive results divided by the total number of trials.
[e]POD_{CC} = Candidate method confirmed results divided by the total number of trials.
[f]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[g]$dPOD_C$ = Difference between the candidate method presumptive and confirmed POD values.
[h]CRA = Campden BRI Culture Collection, Chipping Campden, UK.

Table 6. Matrix Study Oculer Rapid 930 CS-Check results vs. Reference method results (1)

Matrix	Strain	Contamination level (cfu/g)[a]	N[b]	CS-Check method			Reference method				
				x[c]	POD_C[d]	95% CI[f]	x	POD_R[e]	95% CI	$dPOD_{CR}$[g]	95% CI
UHT whole milk	*Bacillus subtilis* (CRA[h] 16597)	0	5	0	0.00	0.00, 0.43	0	0.0	0.0, 0.43	0.0	-0.43, 0.43
		0.8	20	8	0.4	0.22, 0.61	11	0.55	0.34, 0.74	-0.15	-0.41, 0.15
		4.1	5	5	1.00	0.57, 1.00	5	1.0	0.57, 1.0	0.0	-0.43, 0.43

[a] = Inoculum level per g of sample matrix.
[b]= Number of test potions.
[c]x = Number of positive test portions.
[d]POD_C = Candidate method presumptive results confirmed positive divided by the total number of trials.
[e]POD_R = Reference method confirmed results divided by the total number of trials.
[f]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[g]$dPOD_{CR}$ = POD difference between the candidate method confirmed and the Reference method.
[h]CRA = Campden BRI Culture Collection, Chipping Campden, UK.

REFERENCES CITED

1. Soria, M.C. and Fricker, C., Validation of the Oculer Rapid 930 CS-Check™ for the Determination of Commercial Sterility in Ultria-high Temperature Milk, AOAC *Performance Tested Methods℠* certification number 102301.
2. European Directive 92/46 Annex C Chapter 1.A.4.B for Ultra-high temperature (UHT) milk