Supplementary information

Interlaboratory comparison of endotoxin contamination assessment of nanomaterials

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Short title: Comparing endotoxin contamination assessment of nanomaterials.

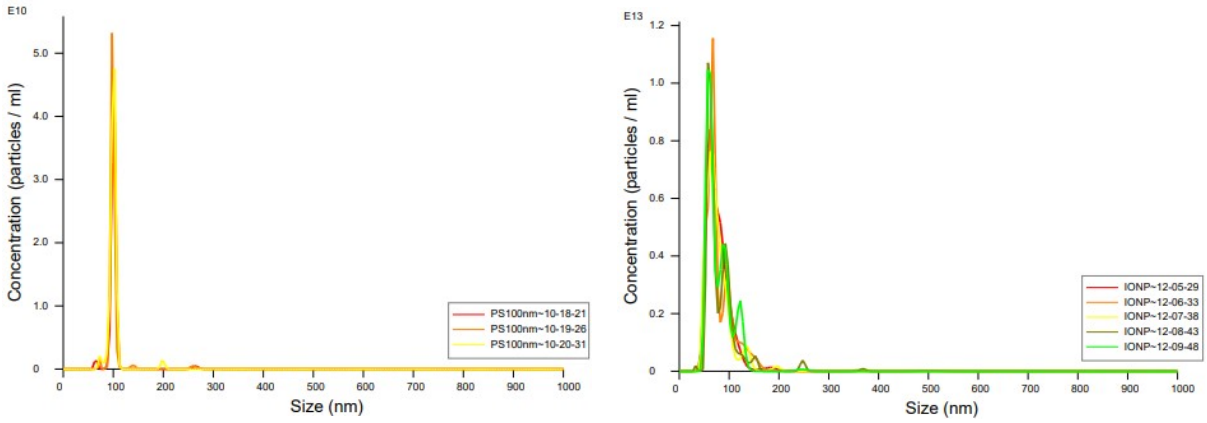
Supplementary Table 1: Suppliers for endotoxin detection kits

Endotoxin detection assays, their manufacturers and component lot numbers and plate readers used are described for each participant in the interlaboratory study. Abbreviation: ACC, Associates of Cape Cod.

Group ID	Assay	Manufacturer	Lot numbers	Plate reader
1	Chromogenic (with beta-glucan inhibition)	ACC	Lysate: 2041801 Endotoxin: H0K354 Glucan inhibition buffer: 1207054	Epoch, Biotek
	Chromogenic (without beta-glucan inhibition)	ACC	Lysate: 2041801 Endotoxin: 166	Epoch, Biotek
	Gel clot (without beta-glucan inhibition)	ACC	Lysate: 518-11-902 Endotoxin: 166	N/A
2	Gel clot (without beta-glucan inhibition)	ACC	Lysate: 518-08-894 Endotoxin: 168	N/A
	Turbidimetric (without beta-glucan inhibition)	ACC	Lysate: 521-03-011-T Endotoxin: 168	Pyros Kinetix Flex, ACC
	Chromogenic (with beta-glucan inhibition)	ACC	Lysate: 2042103 Endotoxin: H0K354 Glucan inhibition buffer: 1207081	Pyros Kinetix Flex, ACC
	Chromogenic (without beta-glucan inhibition)	ACC	Lysate: 2042103 Endotoxin: H0K354	Pyros Kinetix Flex, ACC
3	Chromogenic (with beta-glucan inhibition)	ACC	Lysate: CK0035 Endotoxin: 171 Glucan inhibition buffer: 1207056	Pyros Kinetix Flex, ACC
	Turbidimetric (without beta-glucan inhibition)	ACC	Lysate: 520-06-024-T Endotoxin: 171	Pyros Kinetix Flex, ACC
	Turbidimetric (with beta-glucan inhibition)	ACC	Lysate: 520-06-024-T Endotoxin: 171 Glucan inhibition buffer: 1207056	Pyros Kinetix Flex, ACC
4	Chromogenic (with glucan inhibition)	Pierce	Lysate: 0E10Z00002 Endotoxin: 0H70M55705 Glucan inhibition buffer: 0000988163	ELx800, BIOTEK
	Chromogenic (without glucan inhibition)	Pierce	Lysate: 0E10Z00002 Endotoxin: 0H70M55705	ELx800, BIOTEK
5	Chromogenic (without glucan inhibition)	Thermo Scientific	Lysate: 0000865749 Endotoxin: 0000860495	CLARIOstar Plus, BMG Labtech
	Recombinant factor C	BioMerieux	Factor C: 21555 Endotoxin: 21496	CLARIOstar Plus, BMG Labtech
	Chromogenic (with glucan inhibiton)	Lonza	Lysate: WL088NxM3R Endotoxin: 0000936780 Glucan inhibition buffer: 1207074	CLARIOstar Plus, BMG Labtech
6	Chromogenic (without	Lonza	Lysate: WL028L8LLM	SynergyH1,

	glucan inhibition)		Endotoxin: 0000936780	BIOTEK
	Chromogenic (with glucan inhibition)	Lonza	Lysate: WL028L8LLM Endotoxin: 0000936780 Glucan inhibition buffer: 0000927979	SynergyH1, BIOTEK
7	Chromogenic (without glucan inhibition)	Lonza	Lysate: WL028L8LLM Endotoxin: 0000936780	ELx800, BIOTEK
	Chromogenic (with glucan inhibition)	Lonza	Lysate: WL045J0AAB Endotoxin: 0000904567 Glucan inhibition buffer: 0000988163	ELx800, BIOTEK
8	Recombinant factor C	Lonza	Lysate: 0000961459 Endotoxin: 0000920758	Infinite 200 Pro, TECAN
	Gel clot (without beta-glucan inhibition)	Lonza	Lysate: 0000722832 Endotoxin: 0000723534 Glucan inhibition buffer: 0000988163	N/A

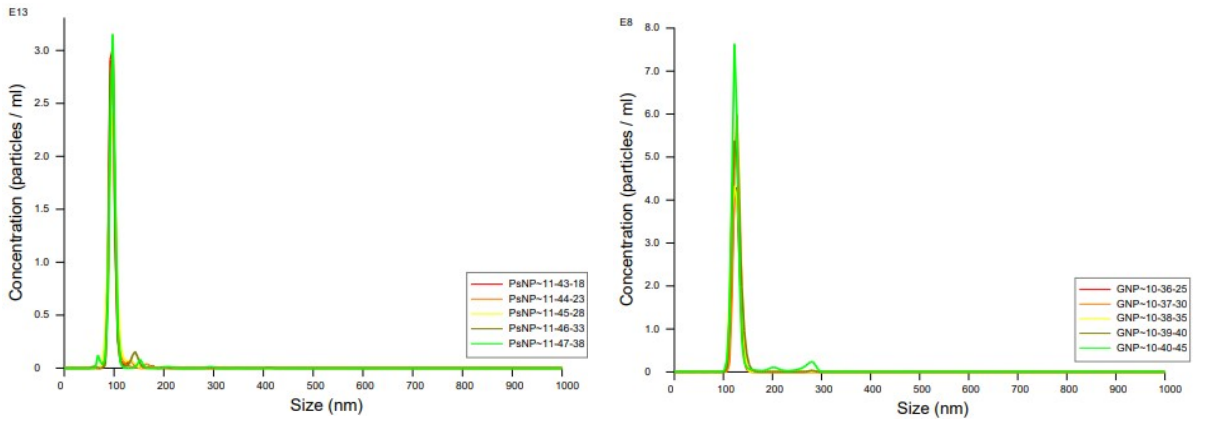
A.



B.

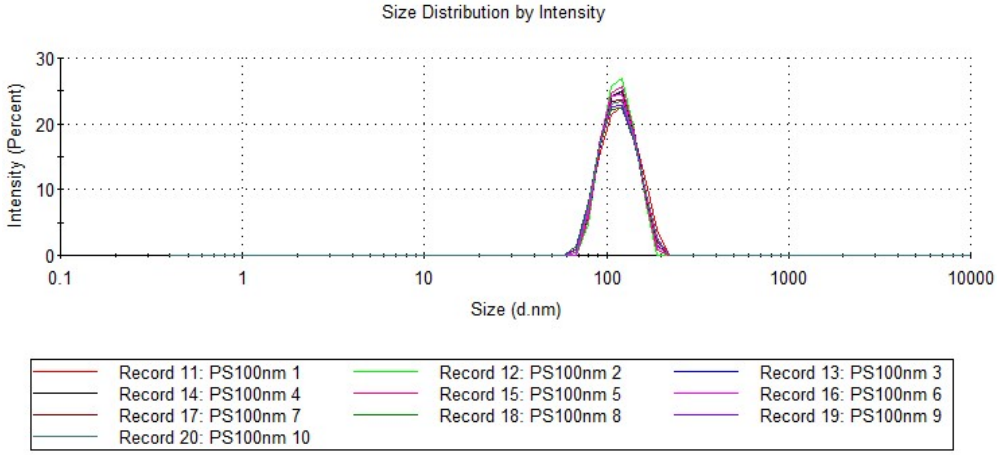
C.

D.

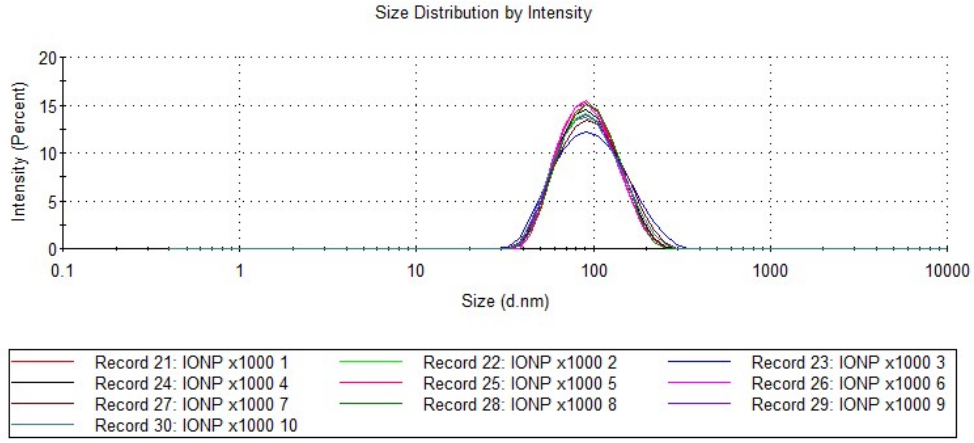


Supplementary Figure 1: Data graphs for hydrodynamic size distribution by nanoparticle tracking analysis by the ILC coordinator prior to shipment

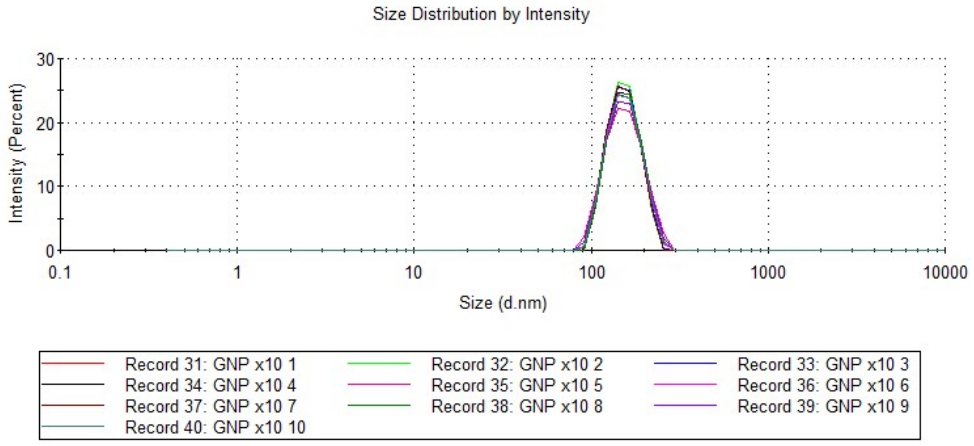
Nanoparticle tracking analysis detailing size distribution for 100 nm calibration standard polystyrene nanoparticles (A), IONP (B), PsNP (C) and GNP (D). X-axis denotes hydrodynamic diameter for each nanomaterial while the Y-axis describes the corresponding nanoparticle concentration (measured in particle/ml).


Size Distribution by Intensity
Intensity (Percent)
30
20
10
0
0.1
1
10
100
1000
10000
Size (d.nm)
Record 11: PS100nm 1
Record 12: PS100nm 2
Record 13: PS100nm 3
Record 14: PS100nm 4
Record 15: PS100nm 5
Record 16: PS100nm 6
Record 17: PS100nm 7
Record 18: PS100nm 8
Record 19: PS100nm 9
Record 20: PS100nm 10

A.

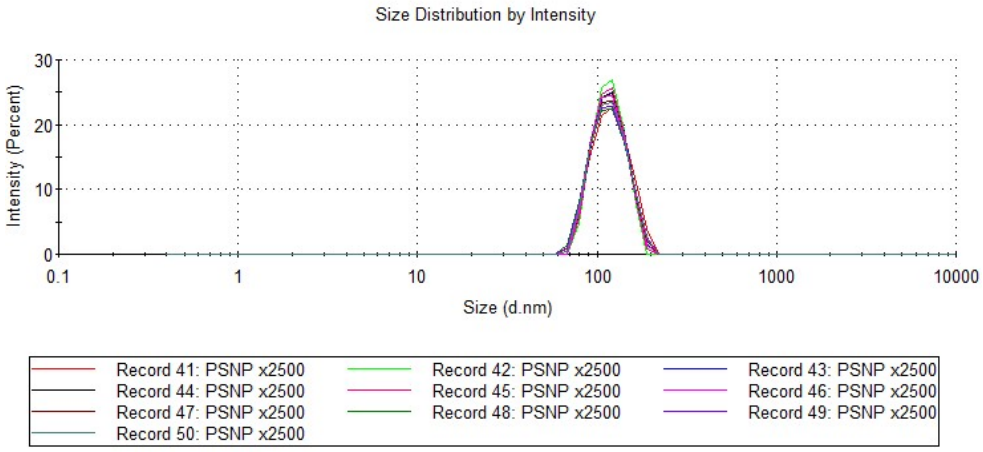

Size Distribution by Intensity
Intensity (Percent)
20
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)
Record 21: IONP x1000 1
Record 22: IONP x1000 2
Record 23: IONP x1000 3
Record 24: IONP x1000 4
Record 25: IONP x1000 5
Record 26: IONP x1000 6
Record 27: IONP x1000 7
Record 28: IONP x1000 8
Record 29: IONP x1000 9
Record 30: IONP x1000 10

B.


Size Distribution by Intensity
Intensity (Percent)
30
20
10
0
0.1
1
10
100
1000
10000
Size (d.nm)
Record 31: GNP x10 1
Record 32: GNP x10 2
Record 33: GNP x10 3
Record 34: GNP x10 4
Record 35: GNP x10 5
Record 36: GNP x10 6
Record 37: GNP x10 7
Record 38: GNP x10 8
Record 39: GNP x10 9
Record 40: GNP x10 10

C.

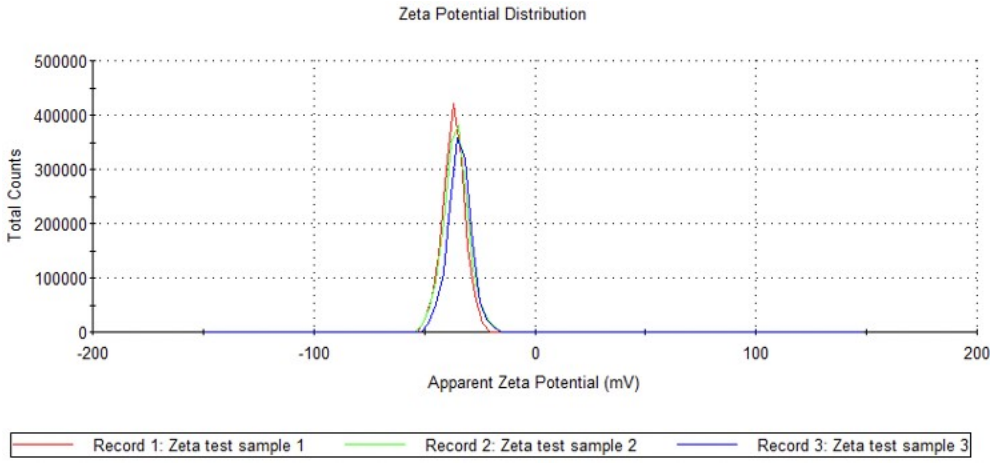
D.



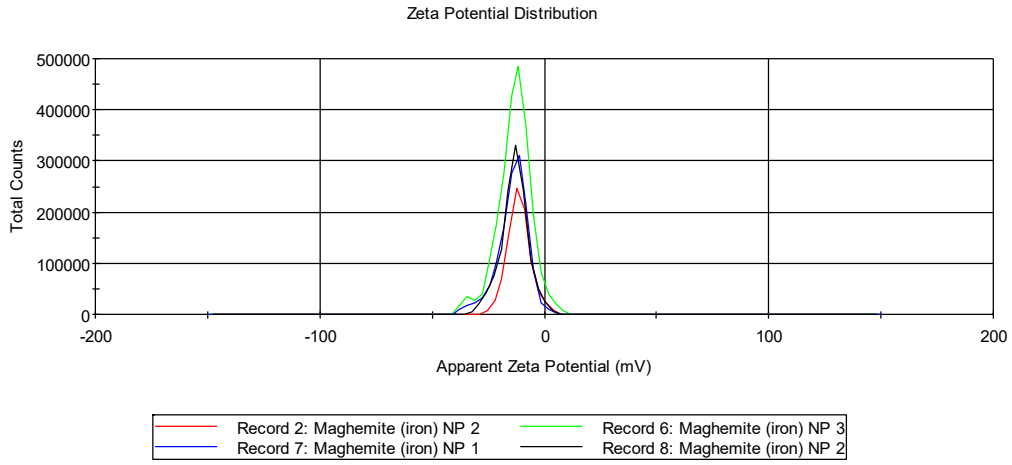
Supplementary Figure 2: Data graphs for hydrodynamic size distribution by dynamic light scattering by the ILC coordinator prior to shipment

Dynamic light scattering detailing size distribution for 100 nm calibration standard polystyrene nanoparticles (A), IONP (B), PsNP (C) and GNP (D). X-axis denotes hydrodynamic diameter for each nanomaterial while the Y-axis describes the light scattering intensity (measured in percent compared to maximum).

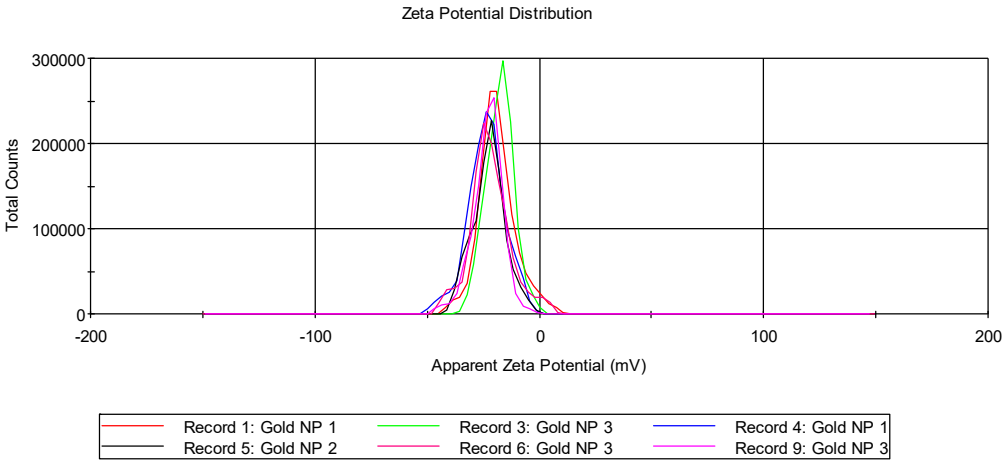
A.


Zeta Potential Distribution
Total Counts
500000
400000
300000
200000
100000
0
-200
-100
0
100
200
Apparent Zeta Potential (mV)
Record 1: Zeta test sample 1
Record 2: Zeta test sample 2
Record 3: Zeta test sample 3

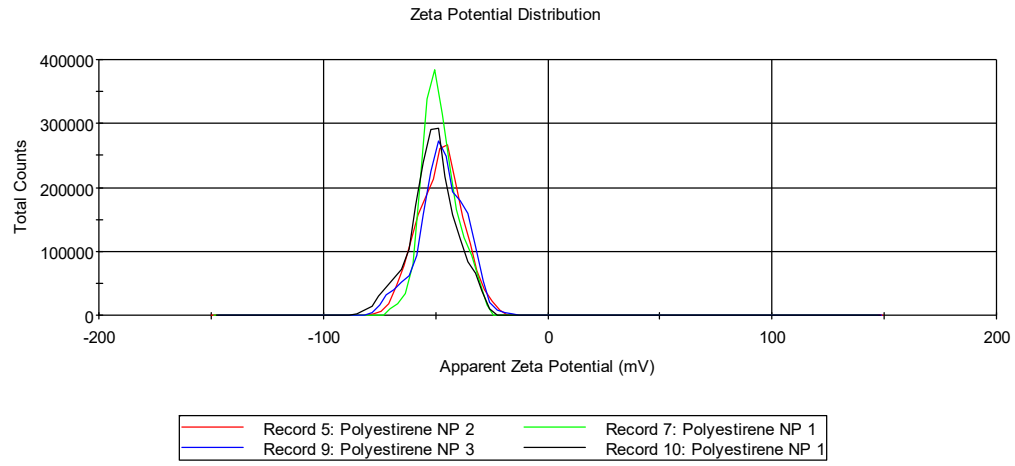
B.


Zeta Potential Distribution
Total Counts
500000
400000
300000
200000
100000
0
-200
-100
0
100
200
Apparent Zeta Potential (mV)
Record 2: Maghemite (iron) NP 2
Record 6: Maghemite (iron) NP 3
Record 7: Maghemite (iron) NP 1
Record 8: Maghemite (iron) NP 2

C.


Zeta Potential Distribution
Total Counts
300000
200000
100000
0
-200
-100
0
100
200
Apparent Zeta Potential (mV)
Record 1: Gold NP 1
Record 3: Gold NP 3
Record 4: Gold NP 1
Record 5: Gold NP 2
Record 6: Gold NP 3
Record 9: Gold NP 3

D.



Supplementary Figure 3: Data graphs for zeta potential measurements by dynamic light scattering

Zeta potential measurements for ZTS1240 Malvern calibration standard (A), IONP (B) at pH 7.5, GNP (C) at pH 7, PsNP (D) at pH 7. and a -35 mV calibration standard (D; Malvern-Panalytical, United Kingdom). X-axis denotes apparent zeta potential while the Y-axis describes the corresponding count of events accounted.

Supplementary Table 2: Summary of results for endotoxin testing

Assays used and corresponding R-squared values or gel clot results are listed from each participant along with inhibition-enhancement (spike-recovery) control percentages or gel clot values. Additionally, the required dilutions used for each sample are recorded along with the associated endotoxin contamination level. Abbreviation: MVD, maximum valid dilution; N/A: not applicable.

Group ID	Assay	R^2 value/ Sensitivity test	IEC %/ result	Dilution required	EU/ml
1	Chromogenic (with beta-glucan inhibition)	$R^2 = 0.99$	IONP: 103% GNP: 116% PsNP: 115% IF: 85%	IONP: x5000 GNP: x20 PsNP: x10000 IF: x2	IONP: 74.97 GNP: 0.31 PsNP: 28.16 IF: 0.002
	Chromogenic (without beta-glucan inhibition)	$R^2 = 0.99$	IONP: 114% GNP: 141% PsNP: 99% IF: 69%	IONP: x5000 GNP: x25 PsNP: x1000 IF: x1	IONP: 22.18 GNP: 0.38 PsNP: 86.98 IF: 0.01
	Gel clot (with beta-glucan inhibition)	Pass	IONP: No value obtained GNP: No value obtained PsNP: No value obtained IF: Value obtained	IONP: MVD reached GNP: MVD reached PsNP: MVD reached IF: x1	IONP: N/A GNP: N/A PsNP:N/A IF: <0.03
2	Turbidimetric (without beta-glucan inhibition)	$R^2 = 0.99$	IONP: 95% GNP: 118% PsNP: 114% IF: 117%	IONP: x12500 GNP: x500 PsNP: x2500 IF: x20	IONP: 122.0 GNP: 1.11 PsNP: 6.07 IF: <0.02
	Chromogenic (with beta-glucan inhibition)	$R^2 = 0.99$	IONP: 66% GNP: 113% PsNP: 198% IF: 119%	IONP: x500 GNP: x20 PsNP: x2500 IF: x20	IONP: 437.0 GNP: 0.96 PsNP: <12.5 IF: <0.02
	Chromogenic (without beta-glucan inhibition)	$R^2 = 0.99$	IONP: 150% GNP: 131% PsNP: 148% IF: 121%	IONP: x2500 GNP: x20 PsNP: x12500 IF: x20	IONP: 600.0 GNP: 0.985 PsNP: 101.0 IF: <0.1
	Gel clot (with beta-glucan inhibition)	Pass	IONP: No value obtained GNP: No value obtained PsNP: No value obtained IF: Value obtained	IONP: MVD reached GNP: MVD reached PsNP: MVD reached IF: x1	IONP: N/A GNP: N/A PsNP:N/A IF: <0.03
3	Turbidimetric (without beta-glucan inhibition)	$R^2 = 0.99$	IONP: 122% GNP: 90% PsNP: 131% IF: 137%	IONP: x10000 GNP: x1 PsNP: x5000 IF: x1	IONP: 958.0 GNP: 0.737 PsNP: 253.5 IF: <0.001
	Chromogenic (with beta-glucan inhibition)	$R^2 = 0.99$	IONP: 130% GNP: 156% PsNP: 105% IF: 129%	IONP: x1000 GNP: x5 PsNP: x5000 IF: x1	IONP: 0.214 GNP: 0.205 PsNP: <25.0 IF: <0.005
	Turbidimetric (with beta-glucan inhibition)	$R^2 = 0.99$	IONP: 130% GNP: 64% PsNP: 130% IF: 183%	IONP: x1000 GNP: x1 PsNP: x2500 IF: x1	IONP: 0.005 GNP: 0.199 PsNP: 0.0598 IF: <0.001
4	Chromogenic	$R^2 = 0.98$	IONP: 75%	IONP: x500	IONP: 292.0

	(without beta-glucan inhibition)		GNP: 86% PsNP: 53% IF: 90%	GNP: x2 PsNP: x1000 IF: x1	GNP: 1.1 PsNP: 706.1 IF: 0.01
	Chromogenic (with beta-glucan inhibition)	$R^2 = 0.99$	IONP: 123% GNP: 109% PsNP: 83% IF: 148%	IONP: x500 GNP: x2 PsNP: x1000 IF: x1	IONP: 132.4 GNP: 1.2 PsNP:12.5 IF: <0.01
5	Chromogenic (without beta-glucan inhibition)	$R^2 = 0.99$	IONP: 100% GNP: 95% PsNP: 91% IF: 108%	IONP: x50 GNP: x1 PsNP: x250 IF: x1	IONP: 44.3 GNP: 0.617 PsNP: 52.25 IF: 0.1
	Recombinant factor C	$R^2 = 0.99$	IONP: 8% GNP: 160% PsNP: 104% IF: 108%	IONP: MVD reached GNP: x1 PsNP: x5 IF: x1	IONP: N/A GNP: 0.148 PsNP: 0.715 IF: 0.01
6	Chromogenic (with beta-glucan inhibition)	$R^2 = 0.99$	IONP: 147% GNP: 131% PsNP: 70% IF: 94%	IONP: x1000 GNP: x5 PsNP: x1250 IF: x1	IONP: 64.19 GNP: 1.1 PsNP: <6.25 IF: <0.005
	Chromogenic (without beta-glucan inhibition)	$R^2 = 0.99$	IONP: 179% GNP: 179% PsNP: 77% IF: 99%	IONP: x1000 GNP: x5 PsNP: x1250 IF: x1	IONP: 348.5 GNP: 1.17 PsNP: 71.4 IF: <0.005
7	Chromogenic (with beta-glucan inhibition)	$R^2 = 0.99$	IONP: 147% GNP: 131% PsNP: 70% IF: 133%	IONP: x1000 GNP: x5 PsNP: x1250 IF: 1x	IONP: 146 GNP: 0.8 PsNP: <6.25 IF: <0.005
	Chromogenic (without beta-glucan inhibition)	$R^2 = 0.99$	IONP: 179% GNP: 179% PsNP: 77% IF: 99%	IONP: x1000 GNP: x5 PsNP: x1250 IF: x1	IONP: 256 GNP: 1.12 PsNP: 13.1 IF: <0.005
8	Recombinant factor C	$R^2 = 0.99$	IONP: 6% GNP: 121% PsNP: 37% IF: 133%	IONP: MVD reached GNP: x5 PsNP: MVD reached IF: x1	IONP: N/A GNP: 0.18 PsNP: N/A IF: <0.005
	Gel clot (with beta-glucan inhibition)	Pass	IONP: No value obtained GNP: No value obtained PsNP: No value obtained IF: Value obtained	IONP: MVD reached GNP: MVD reached PsNP: MVD reached IF: x1	IONP: N/A GNP: N/A PsNP:N/A IF: <0.06