Naked-eye enumeration of single chlamydia pneumoniae based on light scattering of gold nanoparticle probe

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Figure S1. Micrographs of Giemsa-stained Hep-2 cells with or without *C. pneumoniae* infection.
(A, B, and C) Hep-2 cells without *C. pneumoniae* infection were cultured for 24, 48, and 72 h,
respectively. (D, E, and F) Hep-2 cells with *C. pneumoniae* infection were cultured for 24, 48, and
72 h, respectively. The inclusions containing *C. pneumoniae* were indicated by red arrows. All
images were taken at 100 × magnification.



Figure S2. Dark-field images of the control experiment for *C. pneumoniae* detection. (A) 5 μ g of GNP probes only. (B) *C. pneumoniae* only. (C) *C. pneumoniae* mixed with 5 μ g of unfunctionalized GNPs. Scale bar: 10 μ m in the images and 1 μ m in the upper right corner of each of images.





Figure S3. Melting curve of qPCR assay of *C. pneumoniae*, other control pathogens such as *Salmonella typhimurium, Escherichia coli* and blank control. The data clearly show that no PCR
product produced from control pathogens and blank control.

43 Table S1. The primers for qPCR to amplify 168 bp fragment and the primers and Taqman probe

44 for dPCR to amplify 78 bp sequence of the 23S rRNA gene of *C. pneumoniae*.

The primers for qPCR	Forward: 5'-GGGGTTGTAGGGT CGATAACATGGGATC-3' Reverse: 5'-GAGAGTGGTCTCCCCAGATTCAGACTA-3'
The 168 bp fragment	GGGGTTGTAGGGTCGATAACATGGGATCTTAAGTTTTAGTTG AATACTTCTGGAAAGTTGAACGATACAGGGTGATAGTCCCG TAAACGAAAAAACAAAAGACGCTAATCGATACCTGAGTAGG GCTAGACACGTGAAACCTAGTCTGAATCTGGGGAGACCACT CTC
The primers for dPCR	Forward primer, 5'-CCAAGGTTTCCAGGGTCAAG-3' Reverse primer, 5'-TCCATCGTCTACGCATTTGTG-3' Taqman probe: 5'- FAM-TCTTCCCTGGGTTAGTCGGCCCCTA-3'
The 78 bp fragment	CCAAGGTTTCCAGGGTCAAGCTCGTCTTCCCTGGGTTAGTCG GCCCCTAAGTCGAGGCACAAATGCGTAGACGATGGA

48 Table S2. Standard curve of qPCR assay of C. pneumoniae Concentration of plasmid with 168 bp fragment of the 23S rRNA gene (GeneBank 2×10^8 2×10^7 2×10^6 2×10^5 2×10^4 2×10^3 2×10^2 2×10^1 No: NR_076161) of C. pneumoniae (Copies/µL) Ct values 14.85 17.98 21.69 25.38 28.82 32.02 35.65 38.05 49 50 51 52 53 54 55 Table S3. LOD of qPCR and GNP-labeled dark-field counting method for C. pneumoniae.

	Number of qPCR				Number of GNP-labeled dark-field count						
	(copie	es/µL)				(pathog	gen parti	cle /µL)			
No. sample	1#	2#	3#	4#	5#	1#	2#	3#	4#	5#	6#
Theoretical	8.42	1.68	3.36	1.68	8.4	8.42	1.68	3.36	1.68	8.4	4.2
Concentration	×	×	×	×	×	×	×	×	×	×	×
	102	102	101	101	10^{0}	102	102	101	101	10^{0}	10^{0}
Repeated No.											
NO. 1	827	174	37	19	-	768	180	28	10	2	1
NO. 2	916	152	30	15	-	858	156	21	14	6	2
NO. 3	773	133	26	12	-	782	150	34	18	6	2
Mean	839	153	31	15	-	803	162	28	13	5	2
Standard deviation	72	20	6	4	-	48	16	7	4	2	1

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Figure S4. Curve of PCR assay for cell-profilication *C. pneumoniae* samples at low concentrations
(8.42×10², 1.68×10², 3.36×10¹, 1.68×10¹, and 8.4×10⁰ copies/μL).

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78 Table S4. Capture efficiency of GNP probes for different concentrations of pathogens from cell

79 proliferation.

Dilution samples	Number by GNP-	
at different	labeled dark-field	Capture efficiency
concentrations	counting platform	(%)
(copies/µL)	(particle/µL)	
8.42×10^2	802.7 ± 48	96.5
1.68×10^{2}	162.0 ± 17	98.0
3.36×10^{1}	27.7 ± 7	95.5

80 Statistical analysis: The experimental counting results at different concentrations are reported as the mean \pm SD of triplicate independent experiments. These numbers by GNP-labeled dark-field 81 counting strategy for C. pneumoniae samples are from 10 field of views multiplied by 20 (the area 82 83 of 10 μ L of sample solution in glass slide is almost 200 times that of single field of view). Data are analyzed using the SPSS (Statistical Package for the Social Sciences) software (Version 13.0; SPSS, 84 Inc., Chicago, IL). The capture efficiencies are calculated by the mean values of the samples at these 85 86 certain dilution ratios by our counting strategy divided by those at the same dilution ratios by qPCR 87 method.

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90	Table S5. Enumeration of 10-fold dilution samples of two mice lungs solutions by GNP-labeled
91	dark-field counting method.

	Mouse 2			Mouse 3			
Mathad	aDCD	dPCR	Counting	aDCD	dPCR	Counting	
Methou	YPCK		method	YPCK		method	
Sample NO. 1	480	489	514	695	701	690	
Sample NO. 2	495	506	422	677	688	618	
Sample NO. 3	465	520	464	660	712	650	
Mean	480	505	467	677	700	653	
Standard deviation	15	15.5	46	17.5	12	36	