

Development of label-free gold nanoparticles based rapid colorimetric assay for clinical/point-of-care screening of cervical cancer

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Supporting Information

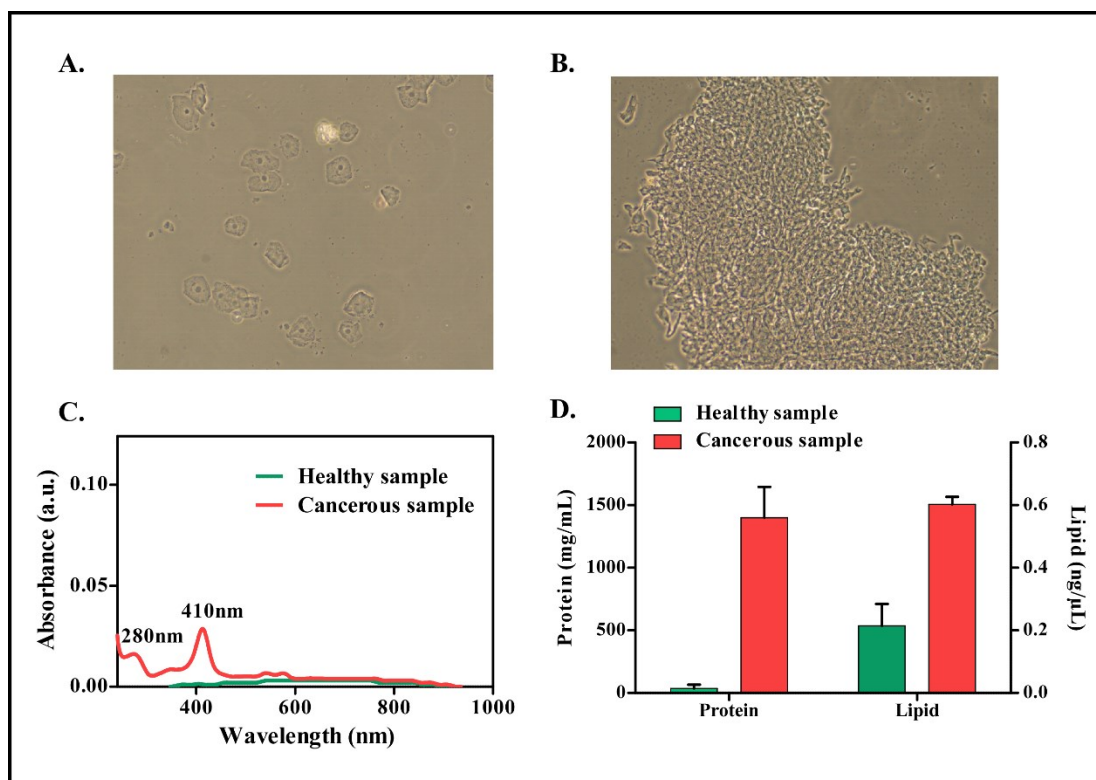


Figure S1: Clinical Samples. Microscopic images of A. Healthy & B. Cancerous cervical fluid samples. (Images were captured at 20X magnification), C. Absorbance spectra of the clinical samples, D. Estimation of protein and lipid content in the clinical samples.

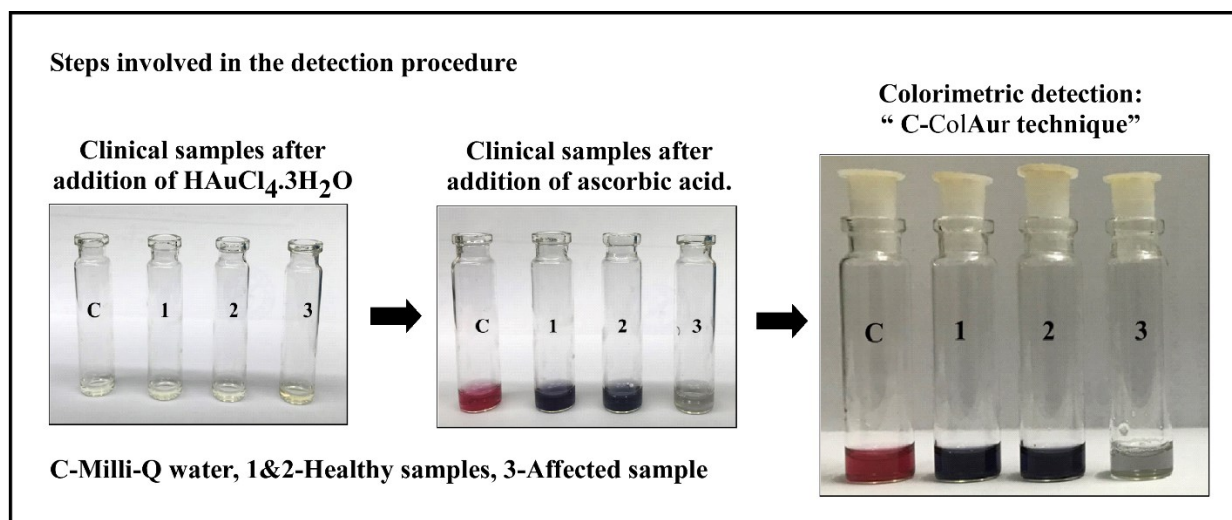


Figure S2: The step by step process of detection of clinical samples.

Table 1: Size and surface charge analysis of blank, control & test Au NPs

Measurement	Au NPs		
	Blank (B)	Control (C)	Test (T)
Size (TEM)	25-40 nm	15-30 nm	250-300 nm
Size (DLS -Volume)	23.4±15.15nm	834.8±611.9nm	252.2±111.48nm
PDI	0.420	0.537	0.195
Zeta Potential (15V)	9.28mV	24.83 mV	-1.08 mV

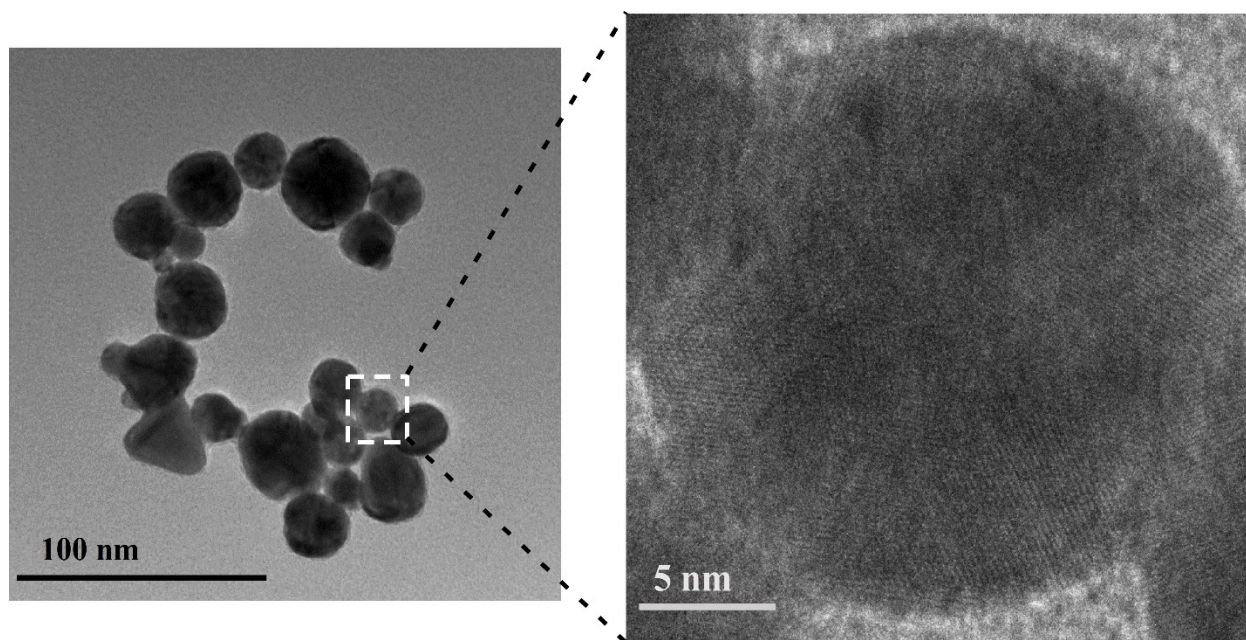


Figure S3: HRTEM imaging of blank Au NPs.

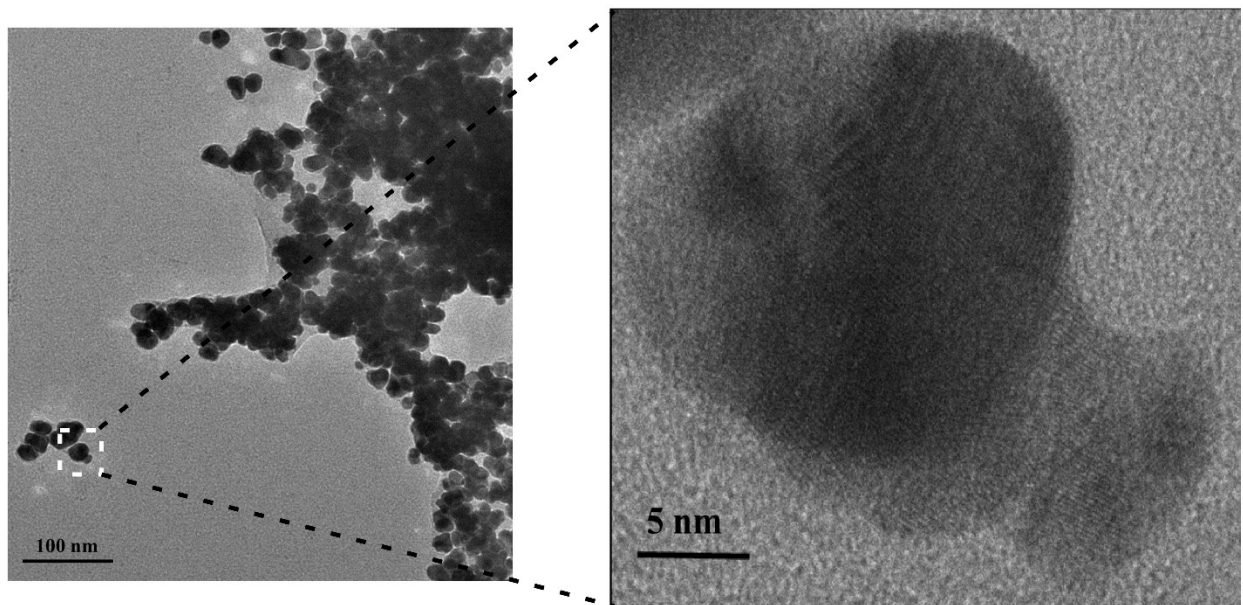


Figure S4: HRTEM imaging of control Au NPs

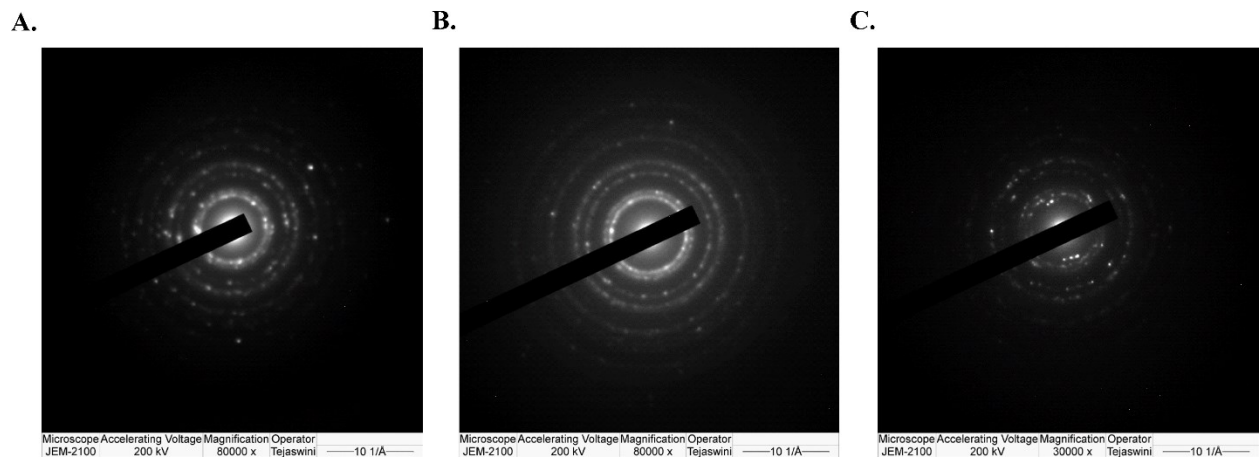
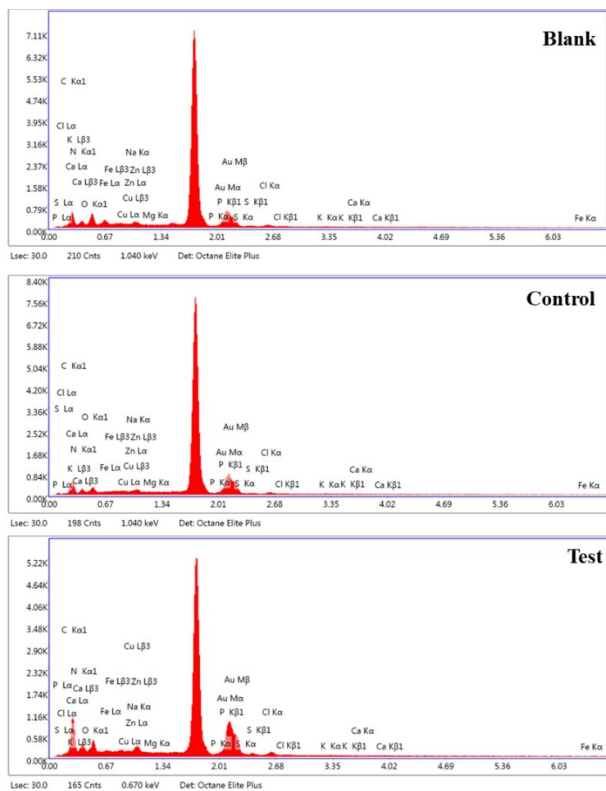


Figure S5: SAED patterns of A) blank, B) control & C) test Au NPs.



Element	Au NPs (Weight %)		
	Blank	Control	Test
C K	11.63	11.89	19.78
N K	4.34	3.21	7.45
O K	10.33	4.96	6.17
Fe L	6.50	0.65	1.62
Cu L	1.49	0.84	1.06
Zn L	2.07	1.40	1.95
Na K	1.75	1.45	1.86
Mg K	0.25	0.31	0.31
P K	0.00	0.00	0.00
Au M	56.18	71.19	56.73
S K	0.01	0.01	0.00
Cl K	3.40	2.66	2.62
K K	0.70	0.80	0.27
Ca K	1.37	0.64	0.18

Figure S6: EDS analysis of A) blank, B) control & C) test Au NPs.

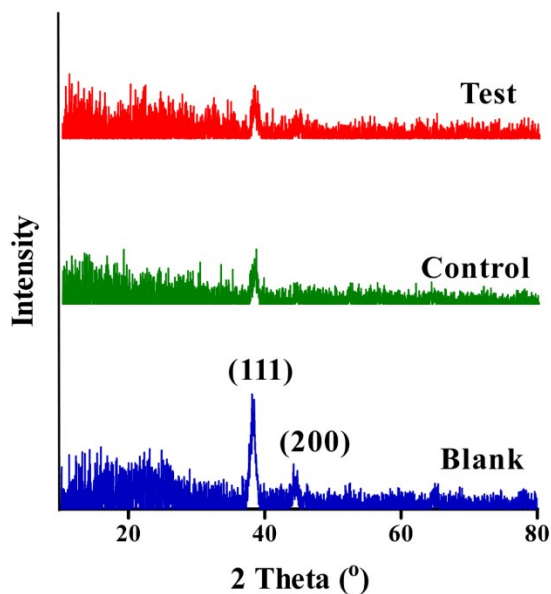


Figure S7: XRD analysis of blank, control & test Au NPs.

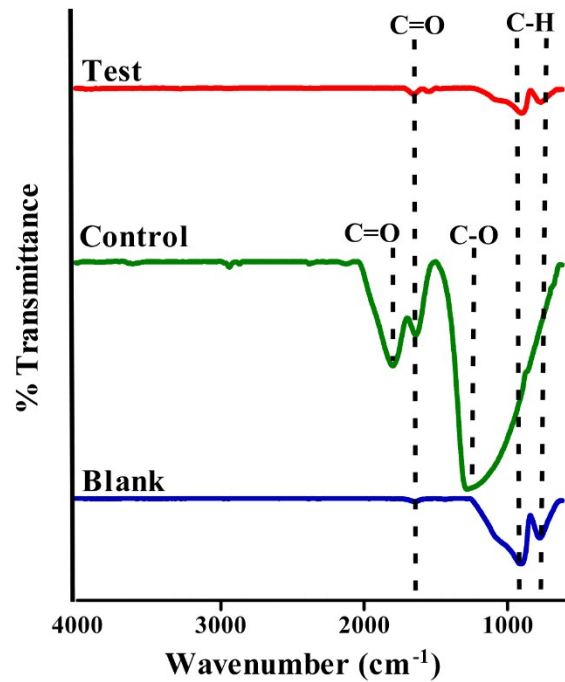


Figure S8: FTIR analysis of blank, control & test Au NPs.

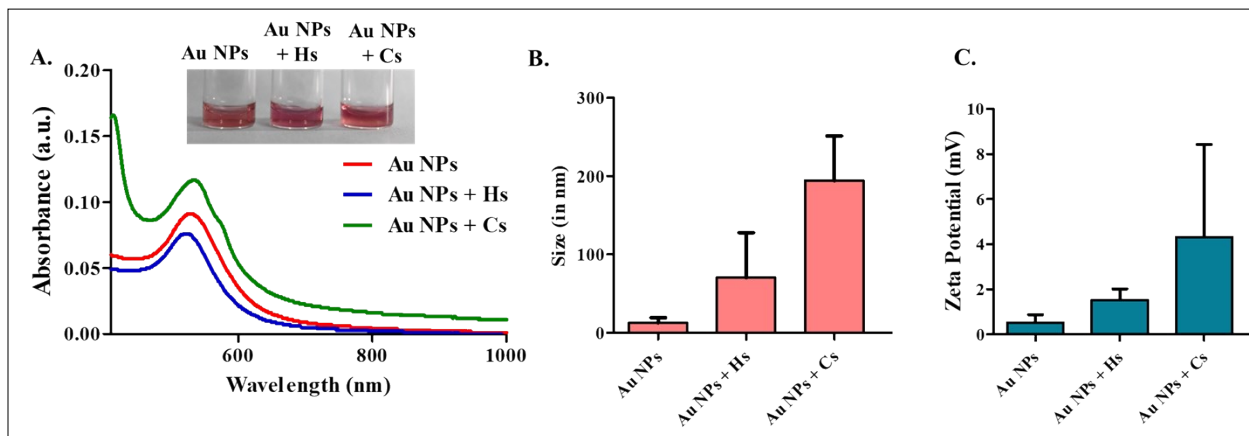


Figure S9: A. UV-visible spectra, B. Hydrodynamic size and, C. Zeta potential of Au NPs before and after addition of clinical samples (Hs: Healthy sample, Cs: Cancerous sample). Inset in Figure A shows the Au NPs before and after clinical sample addition.

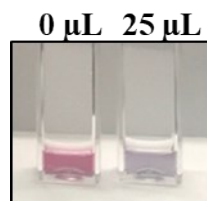


Figure S10: Immediate color change of blank Au NPs with the addition of SSC buffer.

Table 2: Size and surface charge analysis of blank Au NPs treated with SSC buffer.

Measurement	Blank (B) Au NPs	
	0 μ L SSC	100 μ L SSC
Size (Intensity)	76.0 nm	1421.5 nm
Size (Volume)	23.4 nm	3005.2 nm
Size (Number)	11.3 nm	283.7 nm
PDI	0.420	0.885
Zeta (15V)	9.28 mV	10.67 mV

Table 3: Comparison of “C-ColAur” results against gold standard (Pap smear(P)/Biopsy(B)): Healthy Samples

S. No	Date of sample collection	Sample ID	Conventional diagnosis/screening Test (P/B)	Result using conventional test	Cer-ColAur technique
1.	08/05/2019	05/01/01	P	-Ve	-Ve
2.	08/05/2019	05/02/69	P	-Ve	-Ve
3.	08/05/2019	05/03/08	P	-Ve	+Ve
4.	11/05/2019	05/04/TM07	P	-Ve	-Ve
5.	16/05/2019	05/08/TM21	P	-Ve	-Ve
6.	18/06/2019	06/04/TM39	P	-Ve	+Ve
7.	18/06/2019	06/05/TM40	P	-Ve	-Ve
8.	25/06/2019	06/06/TM13	P	-Ve	-Ve

9.	25/06/2019	06/09/TM23	P	-Ve	-Ve
10.	09/07/2019	07/04/MR78	P	-Ve	+Ve
11.	09/07/2019	07/06/MR70	B	-Ve	+Ve
12.	09/03/2018	03/01	P	-Ve	-Ve
13.	10/03/2018	03/03	P	-Ve	-Ve
14.	10/03/2018	03/04	P	-Ve	-Ve

**Table 4: Comparison of “C-ColAur” results against gold standard (Pap smear(P)/Biopsy(B)):
Cancerous samples**

S. No	Date of sample collection	Sample ID	Conventional diagnosis/screening Test (P/B)	Result using conventional test	Cer-ColAur technique
1.	30/04/2019	04/01/MR33	P	+Ve	+Ve
2.	30/04/2019	04/02/MR04	B	+Ve	+Ve
3.	30/04/2019	04/04/MR32	B	+Ve	-Ve
4.	15/05/2019	05/05/MR21	B	+Ve	+Ve
5.	16/05/2019	05/06/MR30	B	+Ve	+Ve
6.	16/05/2019	05/07/30	B	+Ve	+Ve
7.	21/05/2019	05/09/MR59	B	+Ve	+Ve
8.	21/05/2019	05/10/MR47	P	+Ve	+Ve
9.	21/05/2019	05/11/MR73	B	+Ve	+Ve

10.	23/05/2019	05/12/MR72	B	+Ve	+Ve
11.	18/06/2019	06/01/MR96	B	+Ve	+Ve
12.	18/06/2019	06/02/MR09	B	+Ve	+Ve
13.	18/06/2019	06/03/MR49	B	+Ve	+Ve
14.	25/06/2019	06/07/MR77	B	+Ve	+Ve
15.	25/06/2019	06/08/MR76	B	+Ve	+Ve
16.	02/07/2019	07/01/MR70	B	+Ve	+Ve
17.	09/07/2019	07/02/MR51	B,D	+Ve	+Ve
18.	09/07/2019	07/03/MR62	B	+Ve	+Ve
19.	09/07/2019	07/05/MR82	B	+Ve	+Ve
20.	16/07/2019	07/07/MR36	B	+Ve	+Ve
21.	16/07/2019	07/08/MR62	B	+Ve	+Ve
22.	16/07/2019	07/09/MR31	B	+Ve	+Ve
23.	16/07/2019	07/10/MR47	B	+Ve	+Ve
24.	23/07/2019	07/11/MR98	B	+Ve	+Ve
25.	20/08/2019	08/01/MR56	B	+Ve	+Ve
26.	27/08/2019	08/02/MR11	B	+Ve	+Ve
27.	09/03/2018	03/02	B,D	+Ve	+Ve
28.	10/03/2018	03/05	B,D	+Ve	+Ve

*Either Pap test (P) or biopsy (B) were performed in the hospital depending on prior diagnosis. For patients who already had a PAP smear were diagnosed by biopsy. For few patients, diagnosis was performed and doctor's observation (D) was considered as conventional diagnosis.

Table 5: Calculation of sensitivity and specificity for pre-treatment samples.

	Gold standard (Disease present)	Gold standard (Disease absent)	
Test Positive	True Positives (a): 27	False positives (b): 4	Total test positives(a+b): 31
Test Negative	False negatives (c):1	True negatives (d): 10	Total test negatives(c+d): 11
	Total diseased (a+c): 28	Total normal (b+d): 14	Total population (a+b+c+d): 42

Sensitivity: $a/(a+c)$: **96.42%**

Specificity: $d/(b+d)$: **71.42%**

Positive Predictive Value: $a/(a+b)$: **87.09 %**

Negative predictive value: $d/(c+d)$: **90.9%**

Table 6: Calculation of sensitivity and specificity for post-treatment samples.

	Gold standard (Disease present)	Gold standard (Disease absent)	
Test Positive	True Positives (a): 3	False positives (b): 2	Total test positives(a+b): 5
Test Negative	False negatives (c): 1	True negatives (d): 14	Total test negatives(c+d): 15
	Total diseased (a+c): 4	Total normal (b+d): 16	Total population (a+b+c+d): 20

Sensitivity: $a/(a+c)$: **75%**

Specificity: $d/(b+d)$: **87.5%**