Role of PCR in Chronic Hepatitis-C

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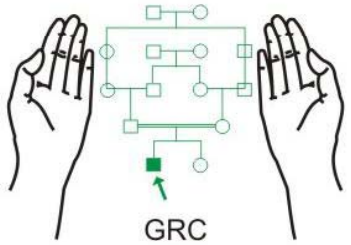
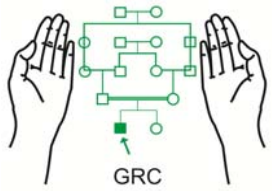
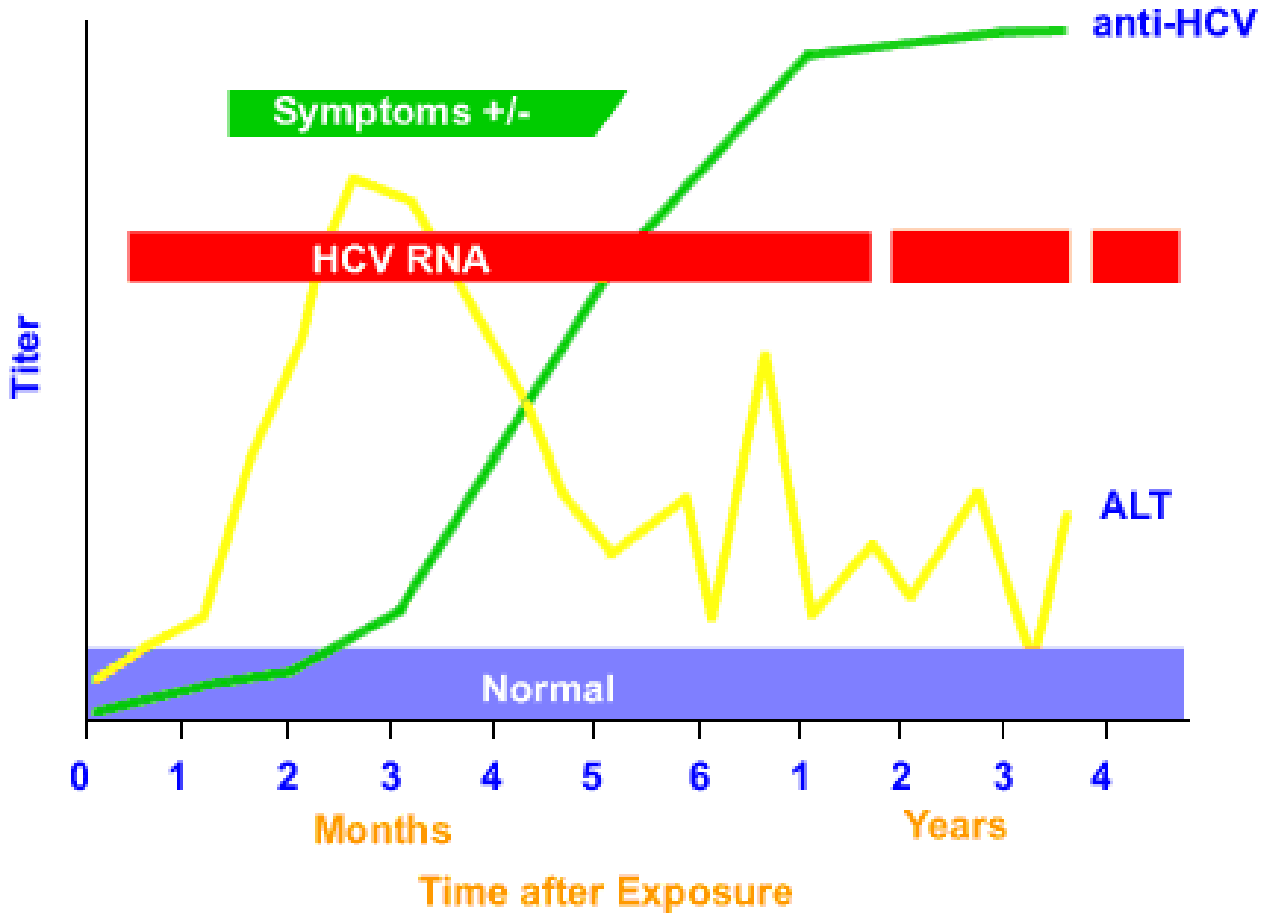


Genetics Resource Centre (GRC)

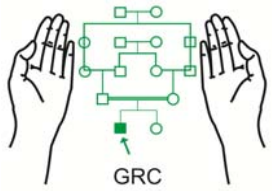
www.grcpk.com



Serologic Pattern of Acute HCV Infection with Progression to Chronic Infection



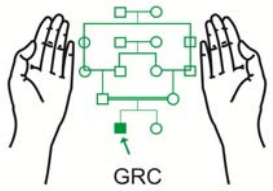
Diagnosis of HCV Infection



- Anti-HCV antibodies are the first line diagnostic test for HCV infection (**recommendation A1**)
- In the case of suspected acute hepatitis C or in immunocompromised patients, HCV RNA testing should be part of the initial evaluation (**recommendation A1**)
- If anti-HCV antibodies are detected, HCV RNA should be determined by a sensitive molecular method (**recommendation A1**)
- Anti-HCV positive, HCV RNA negative individuals should be retested for HCV RNA 3 months later to confirm a recovered infection (**recommendation A1**)

European Association for the Study of the Liver.
EASL Clinical Practice Guidelines: Management of hepatitis C virus infection.
Journal of Hepatology 2014 **60**:392–420.

PCR for HCV



- HCV RNA detection and quantification should be made by a sensitive assay
(lower limit of detection of <15 IU/ml)
(**recommendation A1**)

- The HCV genotype must be assessed prior to treatment initiation and will determine the choice of therapy, the dose of ribavirin and treatment duration
(**recommendation A1**)

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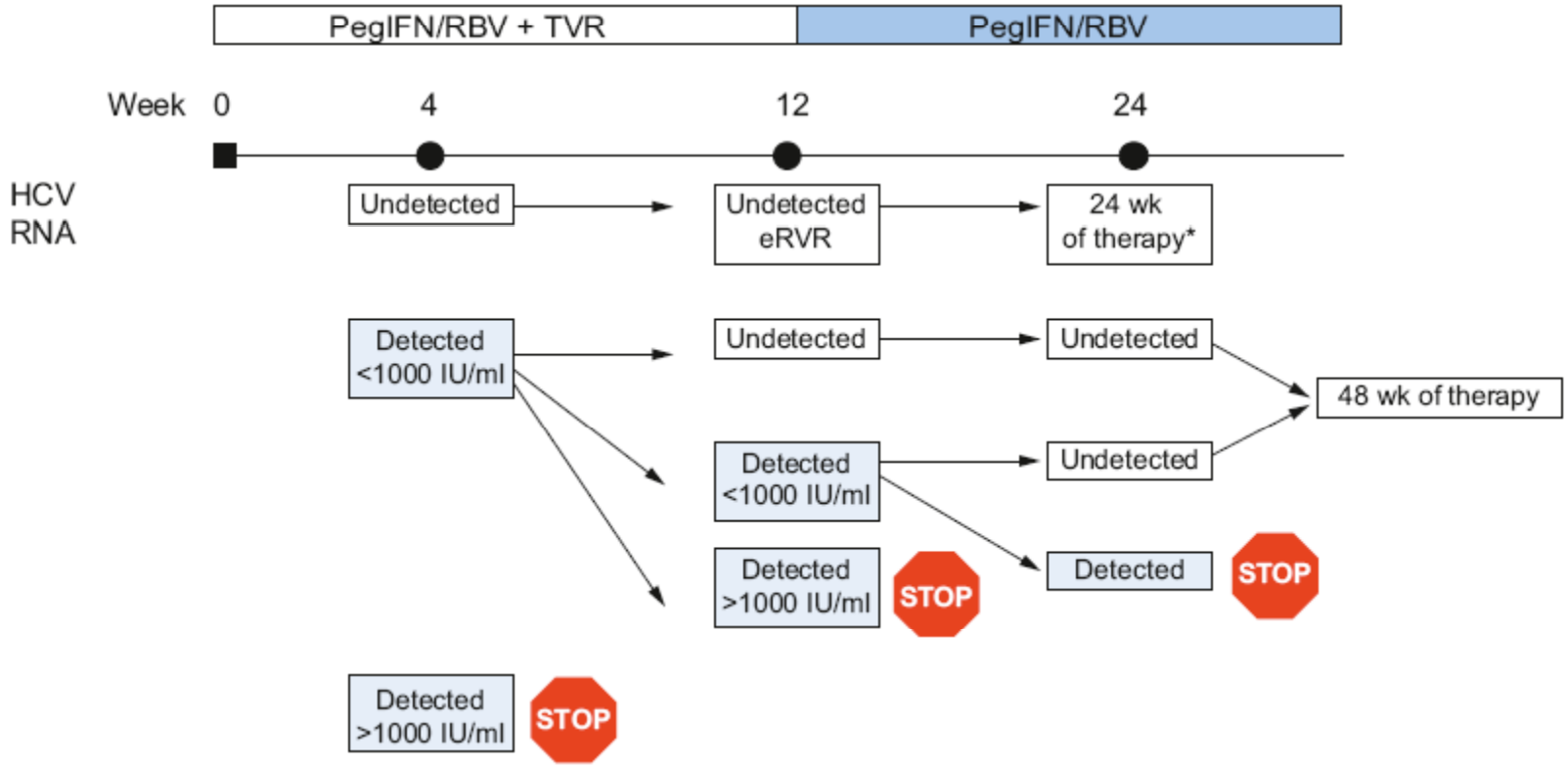
PCR for Monitoring Treatment of HCV



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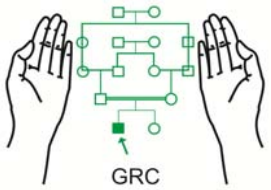
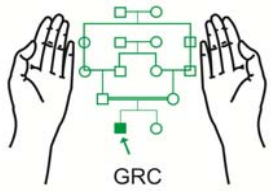
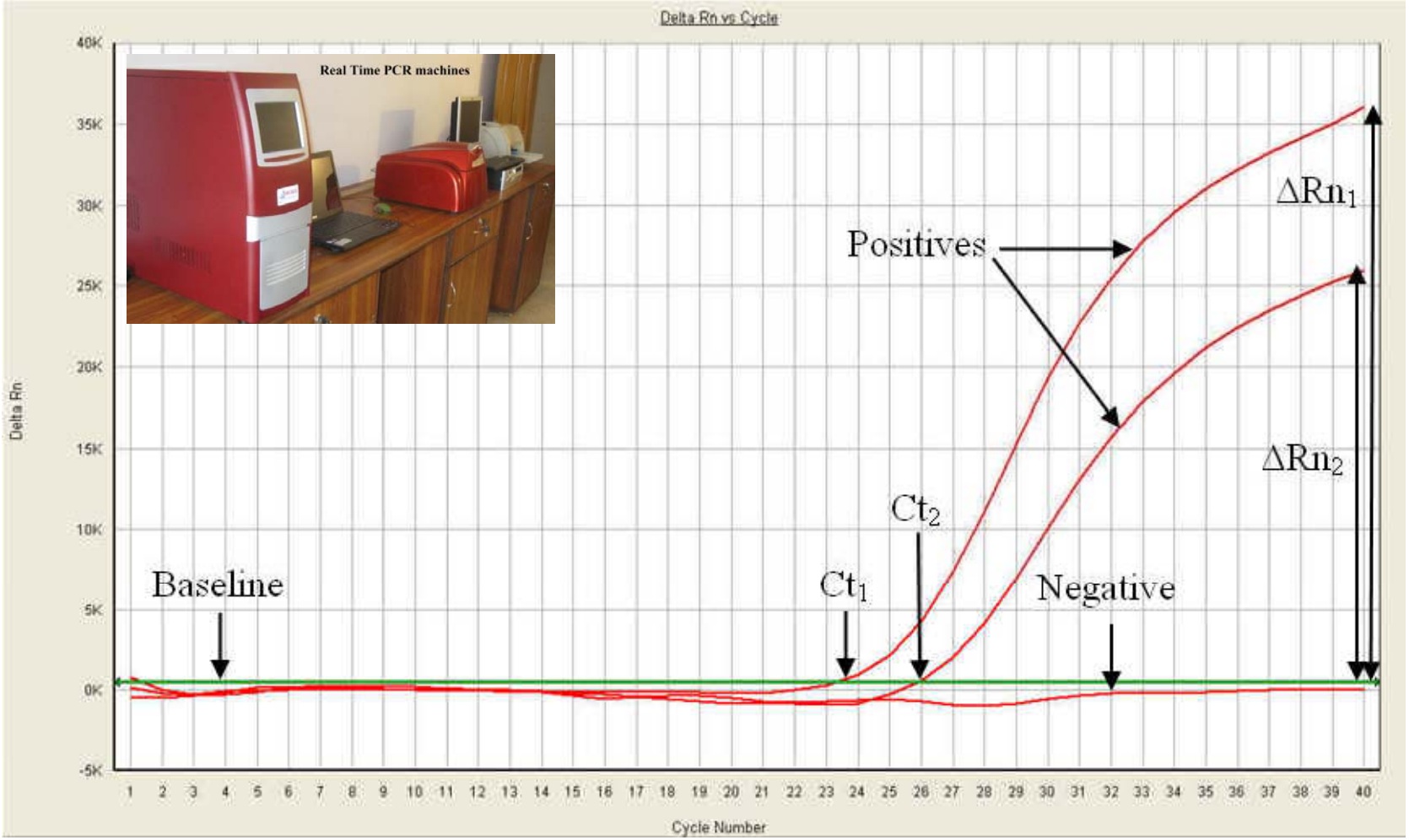


Expectation from HCV PCR

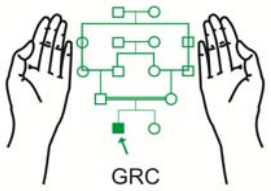


- Detection of 2 log reduction in HCV Viremia
  - 100,000 to <1,000 IU/ml
  - 1000,000 to <10,000 IU/ml
- HCV Viremia <1000 IU/ml
- HCV Viremia <15 IU/ml

Real time PCR

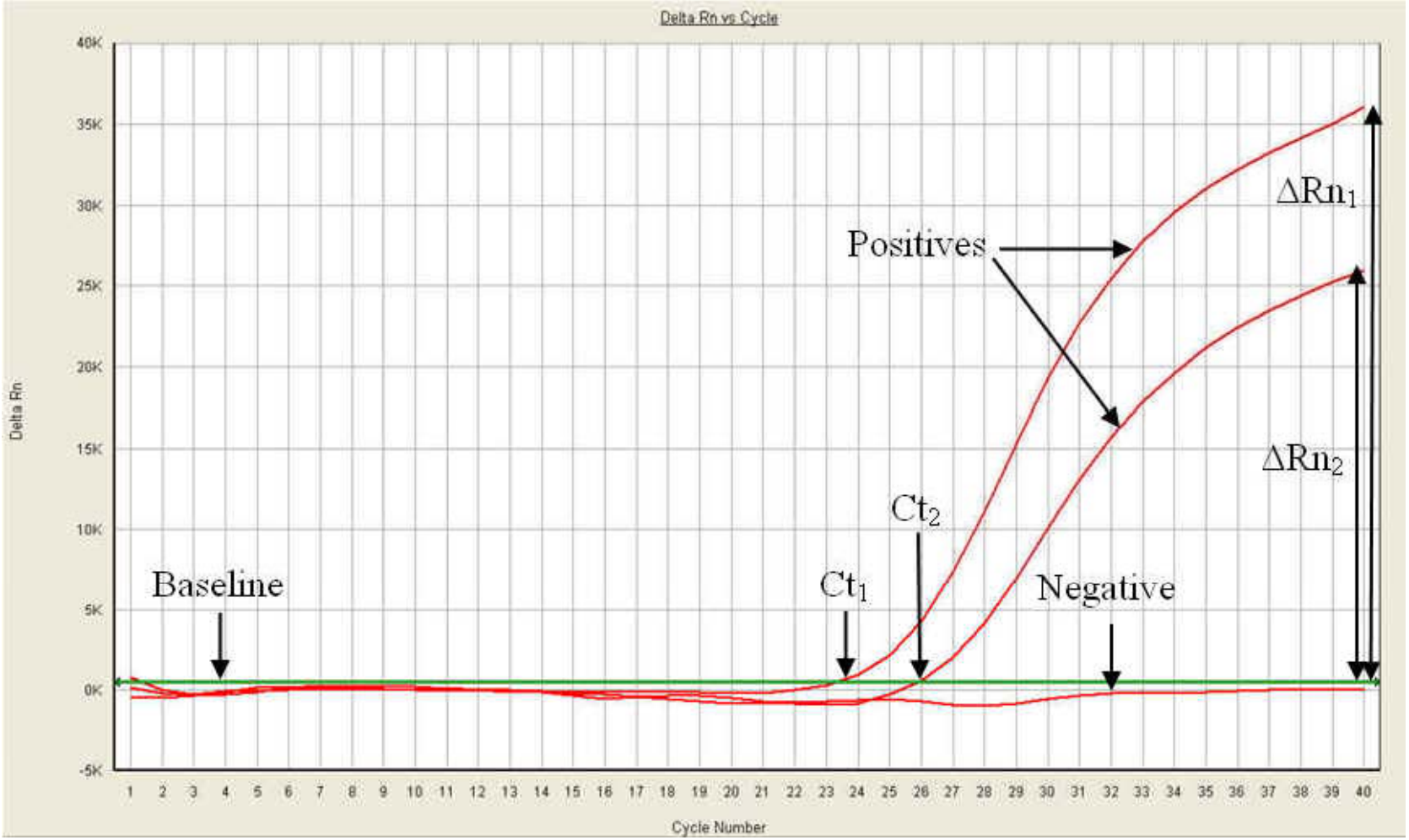


Cost of HCV PCR in Pakistan



Quality	Cost per test	Patient pays
Best	1000-2000	10,000-20,000
Good	500-1000	5000-10,000
Average	<500	3000-5000
None	?	Any price

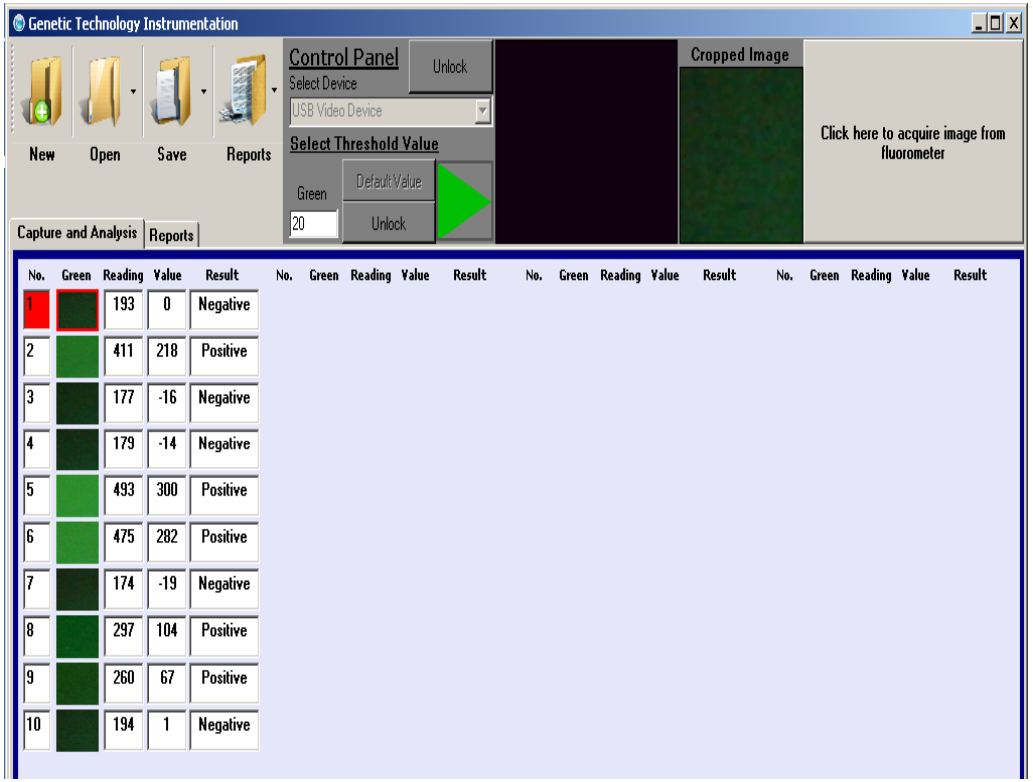
Endpoint Fluorometry
A cheap alternate for Q-PCR



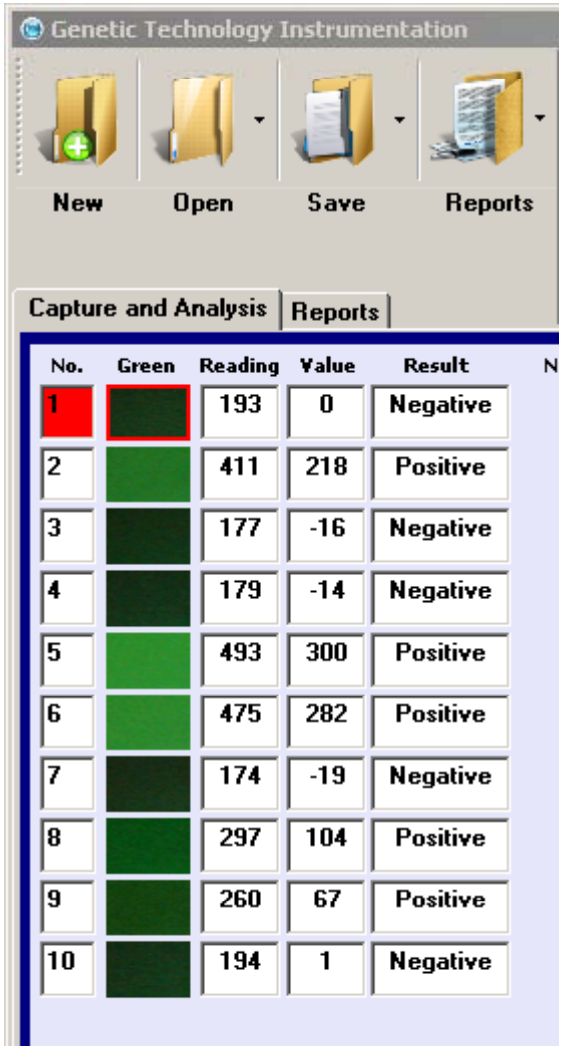
Low-Cost DNA Thermal Cycler for Q-PCR

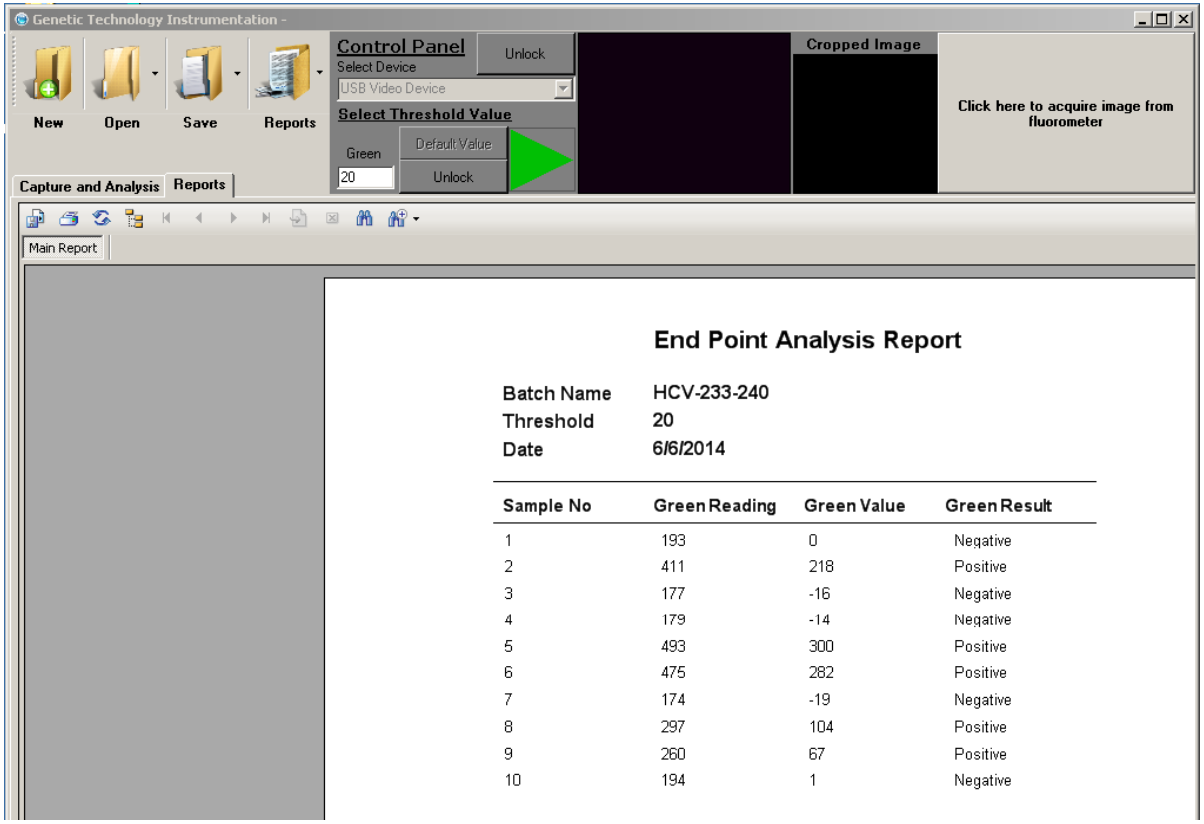
GRC

Endpoint PCR Reader



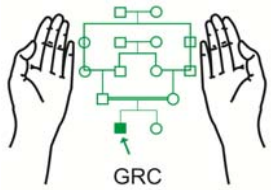
Endpoint PCR Reader





Real time Vs Endpoint Fluorometry

Dil	1/1		1/10		1/100		1/1000		Neg	
Conc IU/ml	1000,000 IU/ml		100,000 IU/ml		10,000 IU/ml		1000 IU/ml		0 IU/ml	
Repeat	**Ct**	**Fluor**	**Ct**	**Fluor**	**Ct**	**Fluor**	**Ct**	**Fluor**	**Ct**	**Fluor**
1	22.3	247	25.1	200	28.1	138	31.4	63	0	4
2	22.1	248	25.3	182	28.1	134	30.9	68	0	8
3	22.1	252	25.2	190	28.1	138	30.7	74	0	1
4	22.7	252	24.8	205	27.8	128	31	79	0	1
5	22.1	261	25	196	27.4	140	31	76	0	5
6	22.3	248	24.8	197	27.5	134	30.1	74	0	1
7	22.1	247	24.8	196	27.3	138	30.1	80	0	2
8	22.2	254	24.9	186	27.6	132	29.9	79	0	4
9	22.5	258	24.7	198	27	133	31.7	61	0	2
10	22.2	249	25	203	27.6	137	30.6	84	0	1
Mean	22.25	251.6	24.96	195.3	27.65	135.2	30.74	73.8	0	2.9
SD	0.21	4.84	0.20	7.29	0.37	3.65	0.58	7.57	0.00	2.33
CV	0.95	1.92	0.78	3.73	1.36	2.70	1.90	10.26		80.38



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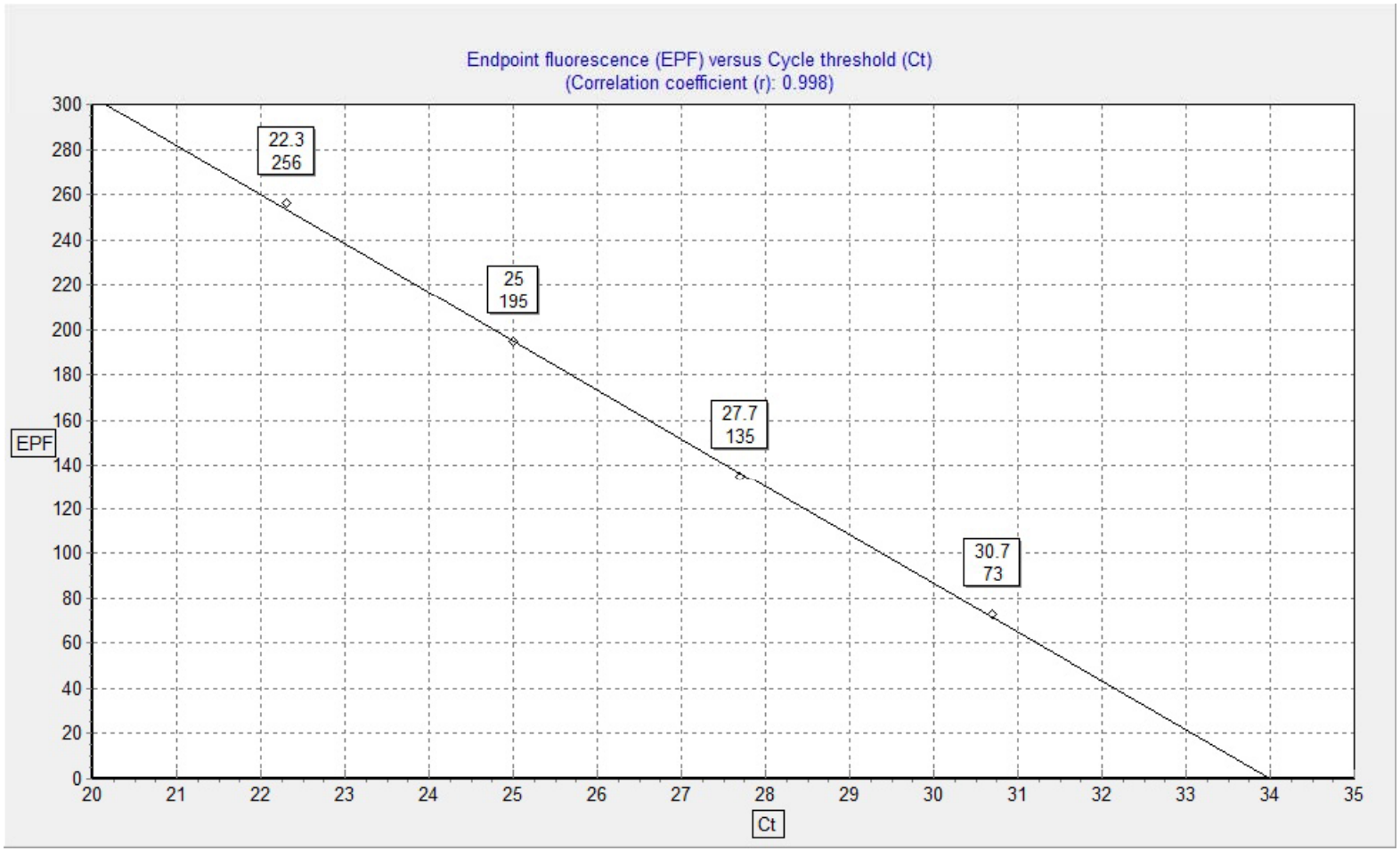

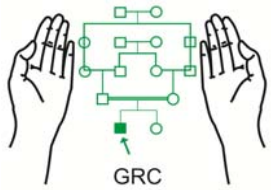

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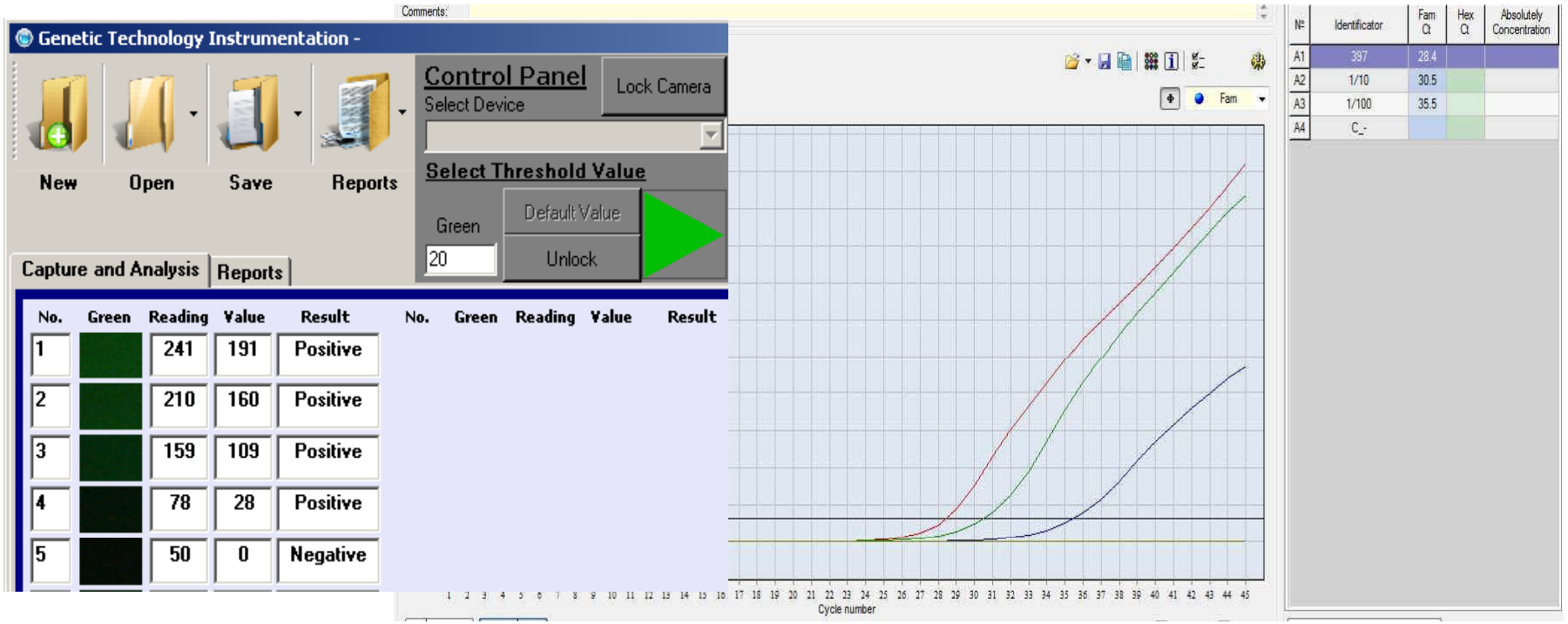

GRC

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