

HBcrAg assay technical updates and applications

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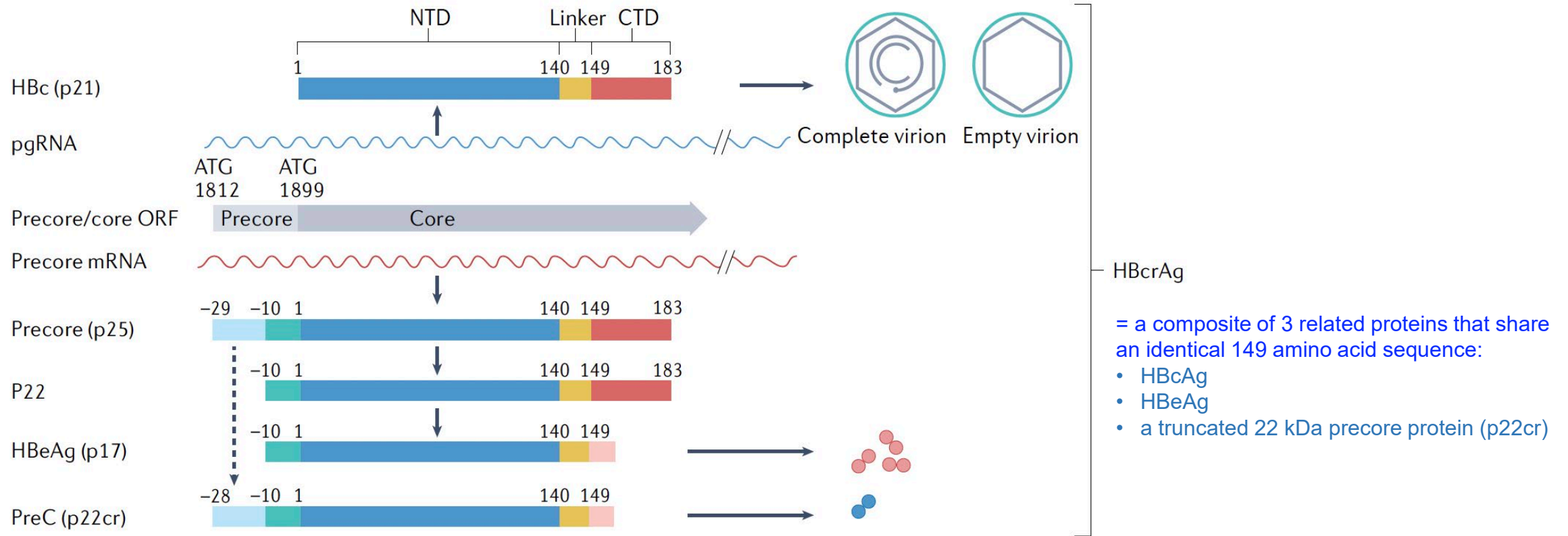


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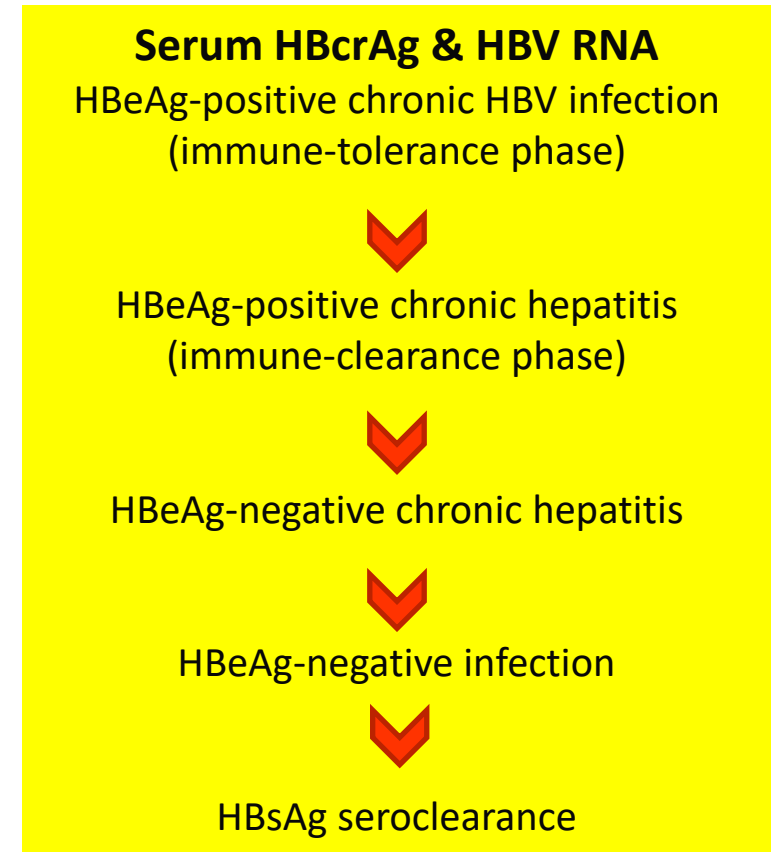
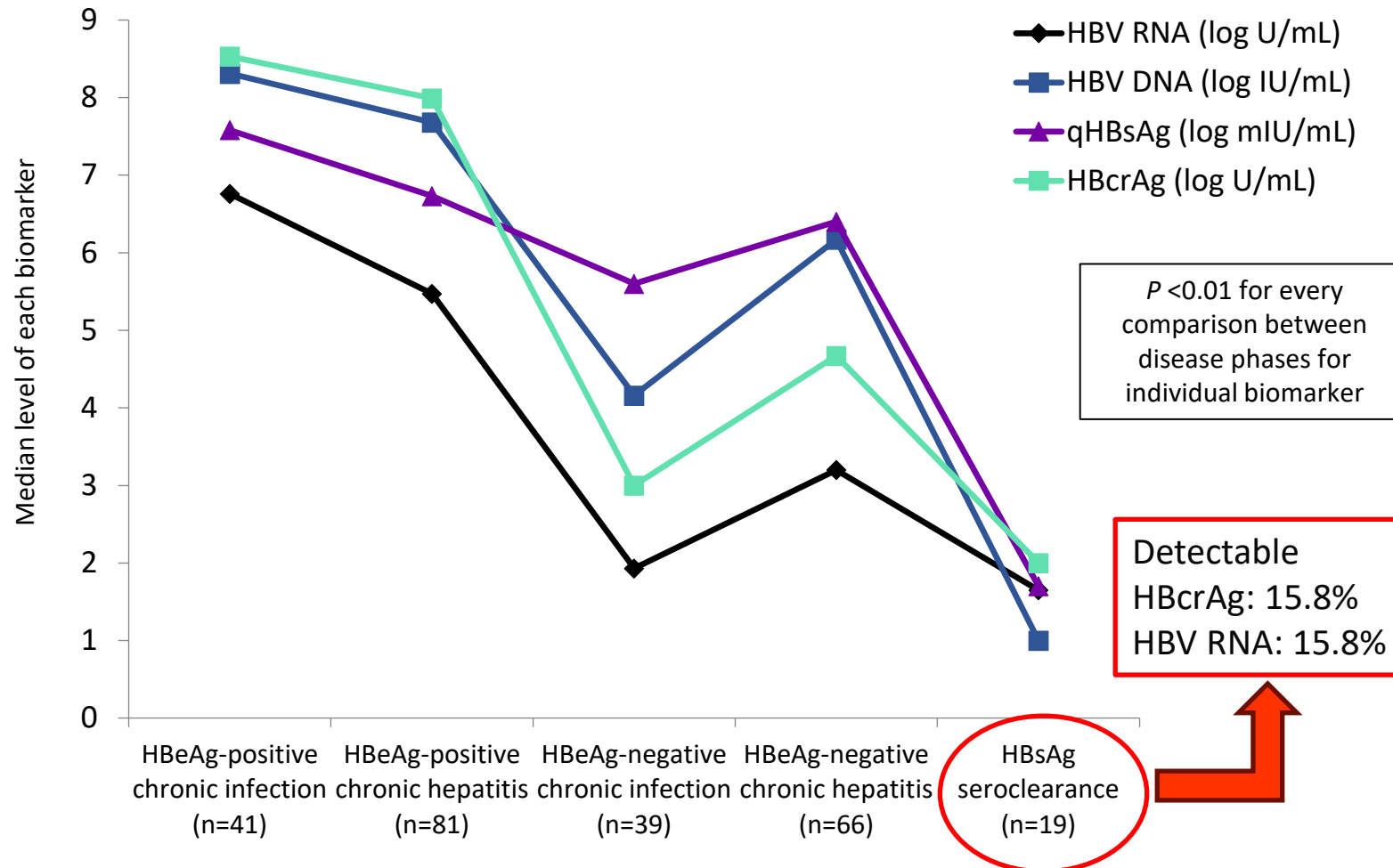
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Hepatitis B core-related antigen (HBcrAg)

Schematic representation of HBcrAg biogenesis



Biomarker levels in different phases of chronic hepatitis B



Detectability of pgRNA and HBcrAg in treatment naïve & NUC-treated patients

Treatment naïve patients

1) HBeAg +ve patients: 100% for both

2) HBeAg –ve patients

	Chronic infection	Chronic hepatitis
HBV RNA	79.5%	97%
HBcrAg	69.2%	87.9%

NUC-treated patients with undetectable HBV DNA

	48 weeks	96 weeks
HBV RNA	87.3%	77.5%
HBcrAg	48.3%	30%

LLOQ for HBV RNA of 44.6 U/mL; HBcrAg of 1000 U/mL
LLOD for HBcrAg 100 U/mL

Two generations of HBcrAg assays

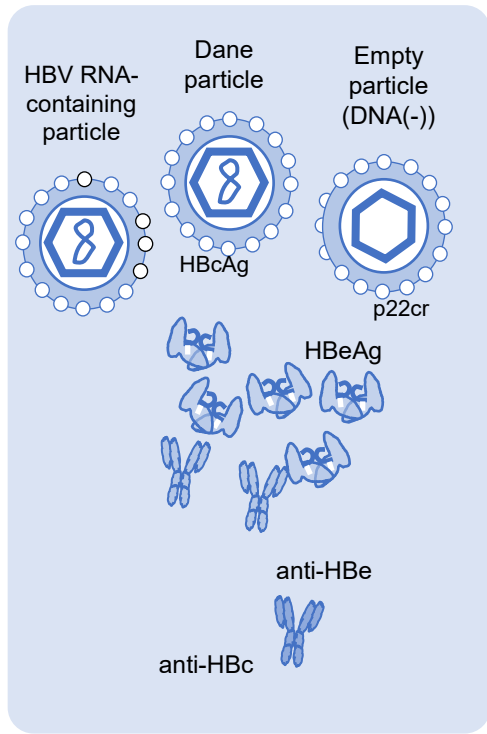
1. Conventional HBcrAg Assay

- On a Lumipulse G machine (named G-HBcrAg)
 - Denaturation of core-related proteins using detergent (Triton X-100)
 - LLOQ 3 log U/mL (1 log U/ml = immunogenicity of 10 pg/mL recombinant HBeAg)

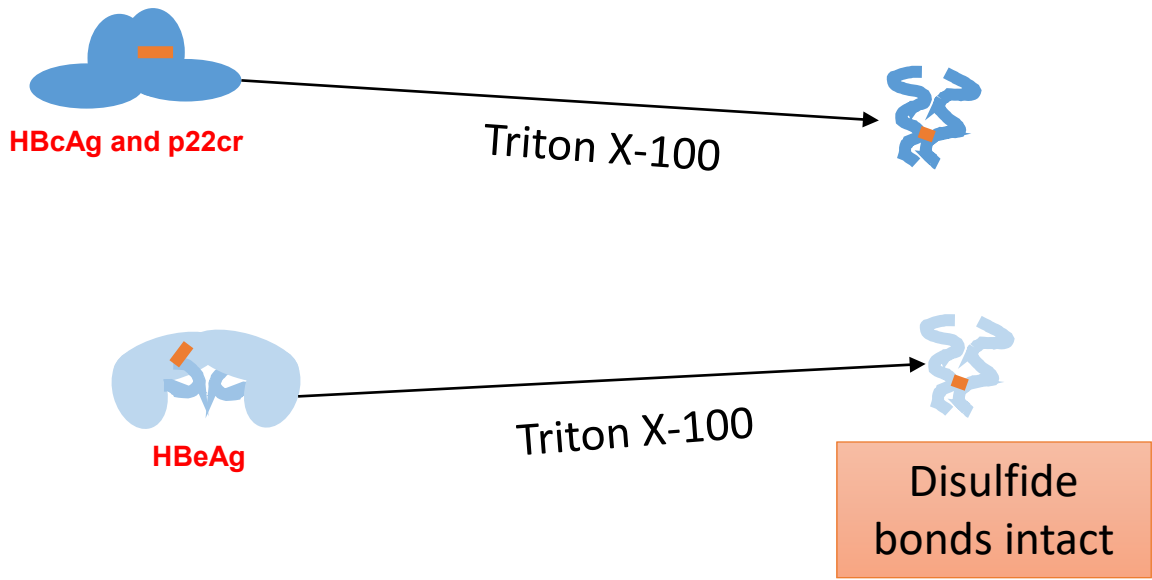
2. New generation- iTACT-HBcrAg (stands for immunoassay for Total Antigen including Complex via pre-Treatment)

- Added a pre-treatment step with acid (HCl) and reducing agent (urea) to disrupt the disulfide bonds
- Increased sensitivity : LLOQ 2.1 log (125.9) U/mL

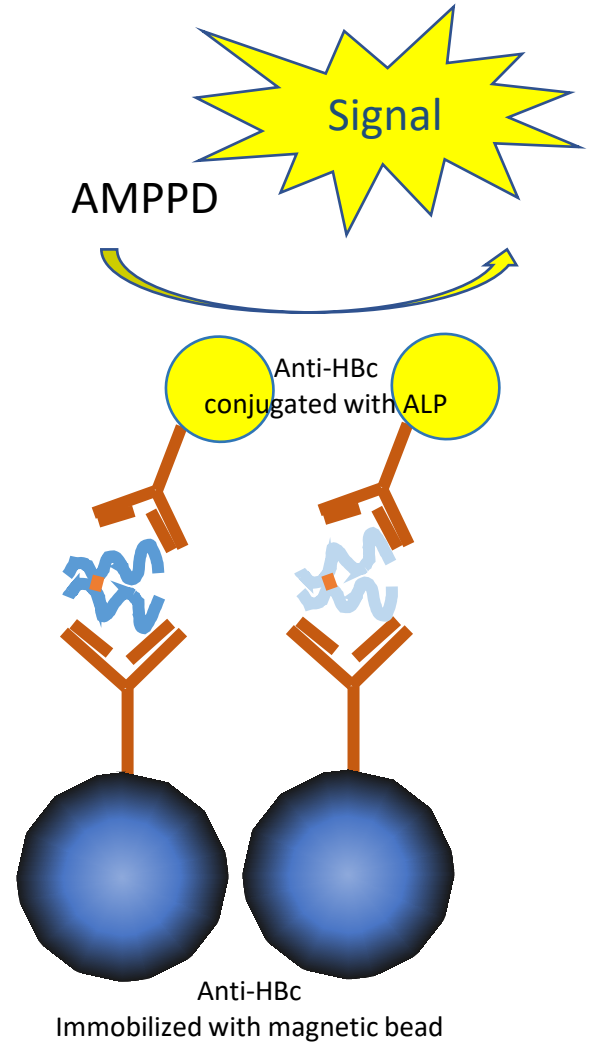
Principle of the G-HBcrAg assay (conventional)



Sample



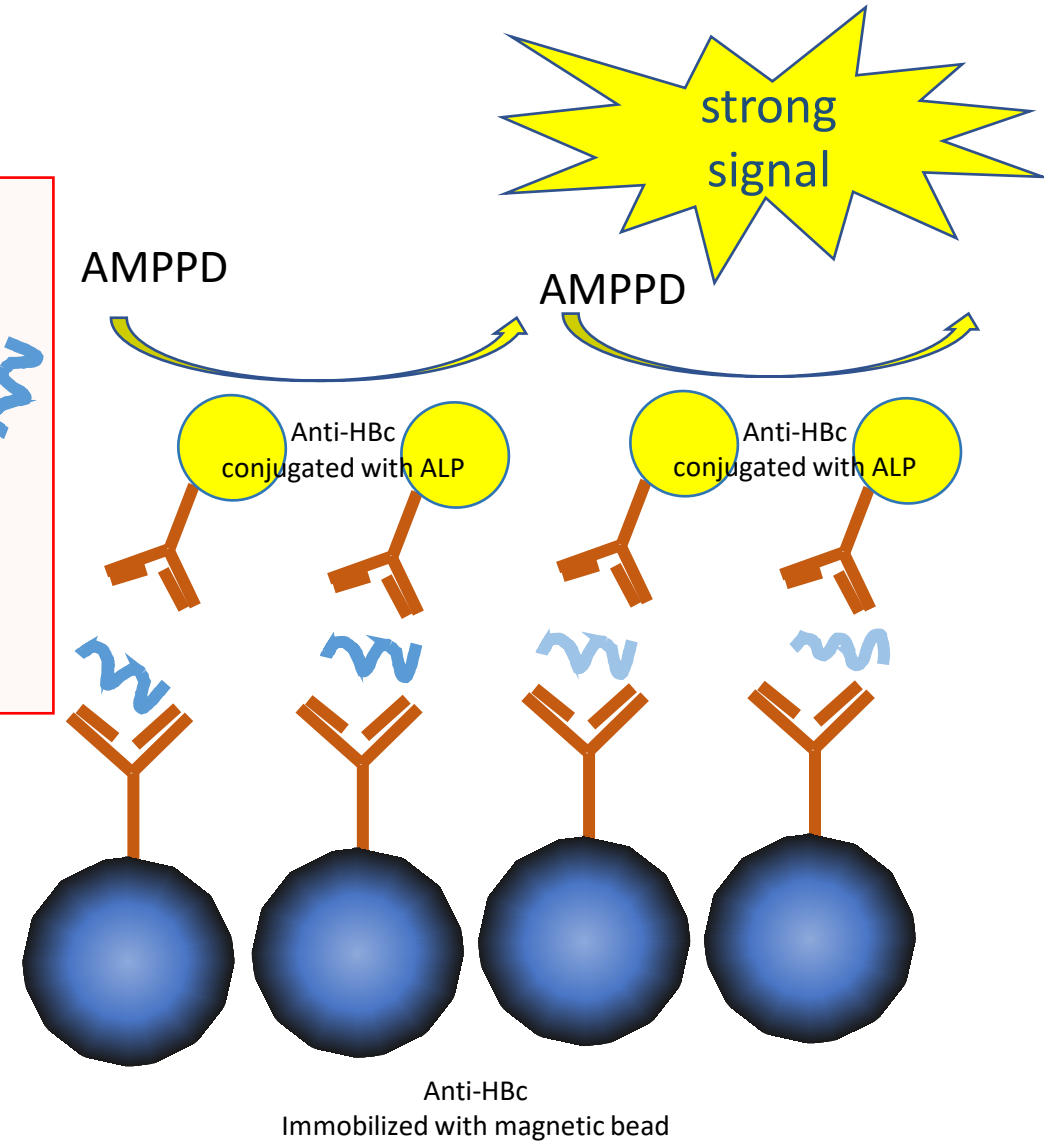
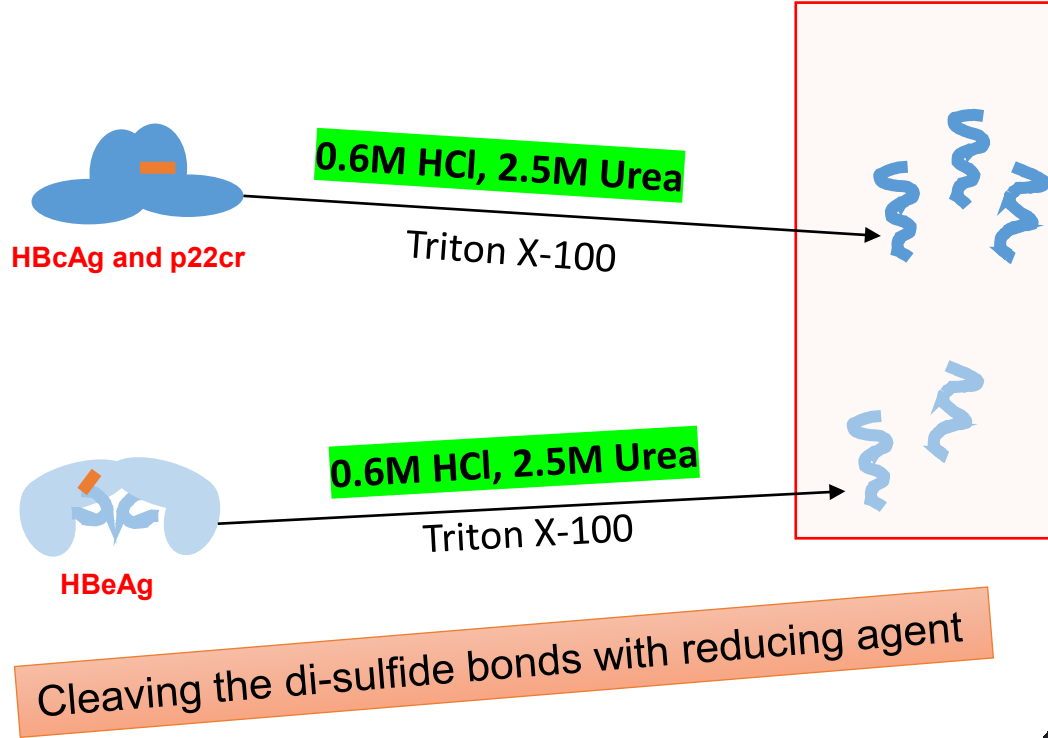
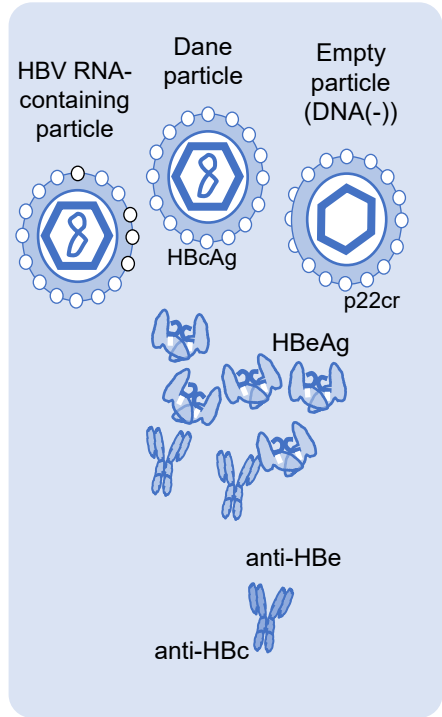
Pretreatment/ denaturation



Detection

AMPPD: 3-(2'- spiroadamantan)-4-methoxy-4-(3'-phosphoryloxy) phenyl-1,2-dioxetane disodium salt

Principle of the iTACT-HBcrAg assay (new)



Sample

Pretreatment/ denaturation

Detection

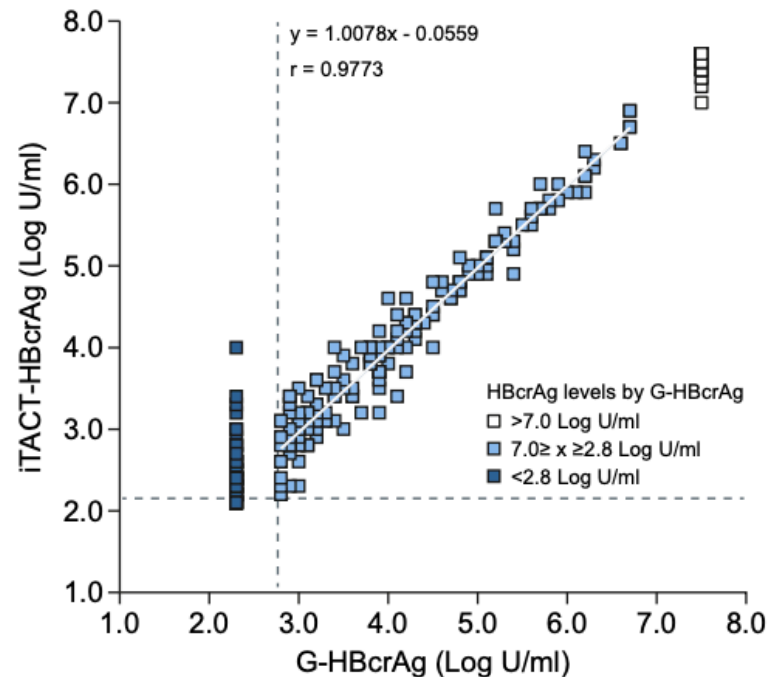
AMPPD: 3-(2'- spiroadamantan)-4-methoxy-4-(3'-phosphoryloxy) phenyl-1,2-dioxetane disodium salt

Detection of HBcrAg using G-HBcrAg and iTACT-HBcrAg assays

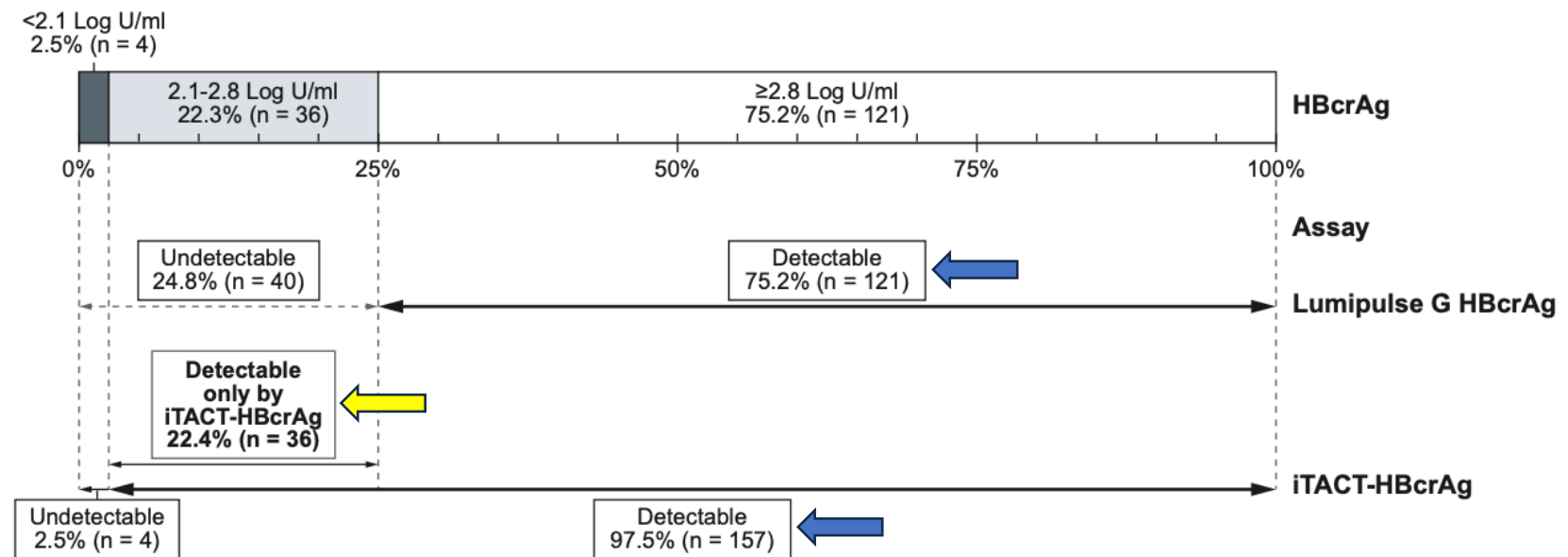
Clinical efficacy of a novel, high-sensitivity HBcrAg assay in the management of chronic hepatitis B and HBV reactivation

Takako Inoue¹, Shigeru Kusumoto², Etsuko Iio³, Shintaro Ogawa³, Takanori Suzuki⁴, Shintaro Yagi⁵, Atsushi Kaneko⁶, Kentaro Matsuura⁴, Katsumi Aoyagi^{5,6}, Yasuhito Tanaka^{1,3,7,*}

Correlation between two assays



Rates of HBcrAg detection by two assays



Detection of HBcrAg in patients with HBsAg seroclearance using G-HBcrAg assay

Hepatol Int (2013) 7:98–105
DOI 10.1007/s12072-012-9354-7

ORIGINAL ARTICLE

Evidence of serologic activity in chronic hepatitis B after surface antigen (HBsAg) seroclearance documented by conventional HBsAg assay

Wai-Kay Seto · Yasuhito Tanaka · Danny Ka-Ho Wong · Ching-Lung Lai ·
Noboru Shinkai · John Chi-Hang Yuen · Teresa Tong · James Fung ·
Ivan Fan-Ngai Hung · Man-Fung Yuen

At 1 year after HBsAg seroclearance, 21% of patients had detectable HBcrAg by the G-HBcrAg assay

LLOQ 3 log U/mL

Seto WK... Yuen MF. *Hepatol Int* 2013;7:98-105

Detection of HBcrAg in patients with HBsAg seroclearance using iTACT-HBcrAg assay

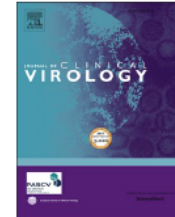
Journal of Clinical Virology 160 (2023) 105375



Contents lists available at [ScienceDirect](#)

Journal of Clinical Virology

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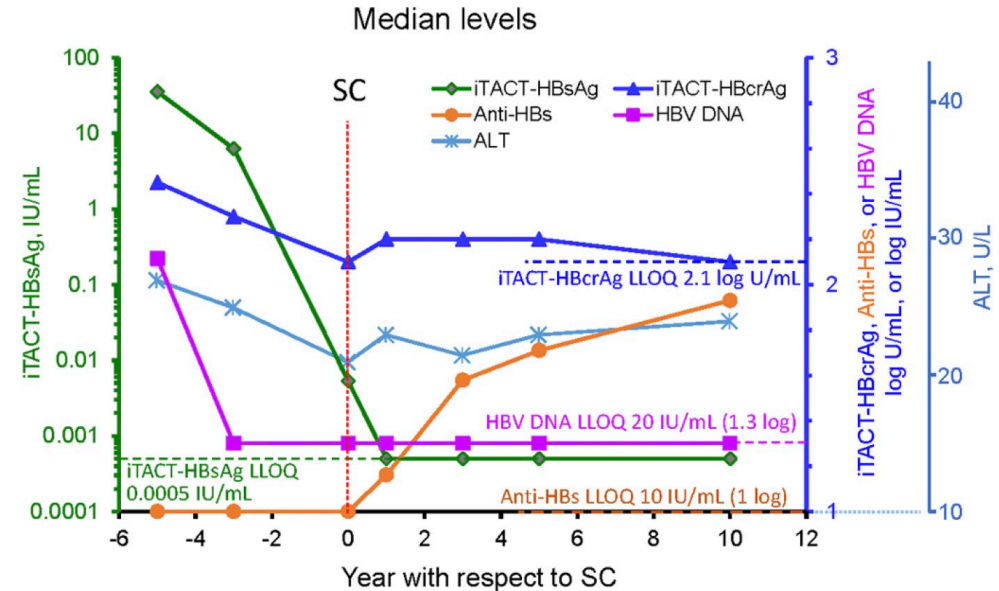
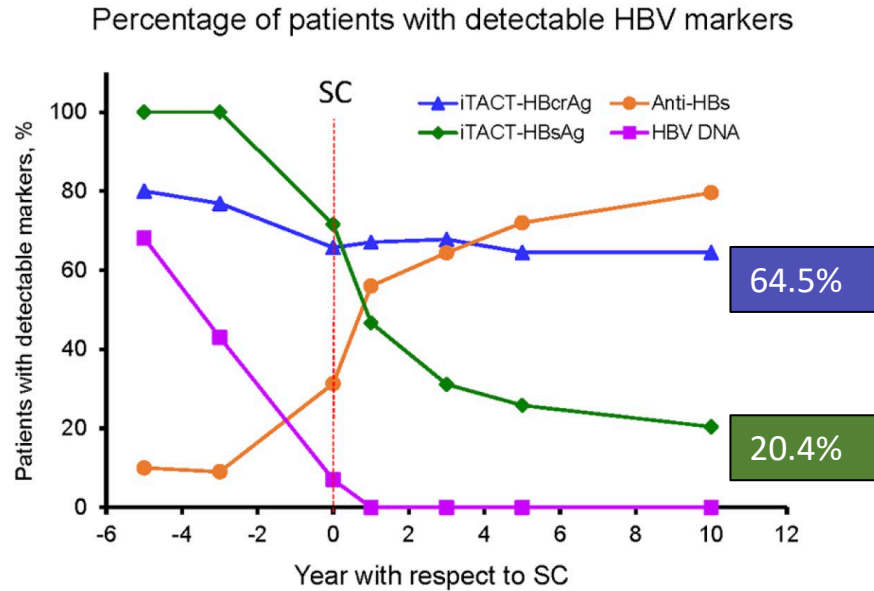
A longitudinal study to detect hepatitis B surface and core-related antigens in chronic hepatitis B patients with hepatitis B surface antigen seroclearance using highly sensitive assays

Danny Ka-Ho Wong^{a,b}, Takako Inoue^c, Lung-Yi Mak^{a,b}, Rex Wan-Hin Hui^a, James Fung^{a,b}, Ka-Shing Cheung^a, Wai-Kay Seto^{a,b}, Yasuhito Tanaka^d, Man-Fung Yuen^{a,b,*}

At 1 year after HBsAg seroclearance, 67.1% of patients had detectable HBcrAg by the iTACT-HBcrAg assay

LLOQ 2.1 log (125.9) U/mL

Detection of HBcrAg/ HBsAg in patients with functional cure using iTACT-HBcrAg & iTACT-HBsAg assays



LLOQ of assays:
 iTACT-HBcrAg 2.1 log (125.9) U/mL
 iTACT-HBsAg 0.0005 IU/mL
 HBV DNA 10 IU/mL

Time after SC, years	SC	1 year	3 years	5 years	10 years
Percentage of samples with detectable:					
iTACT-HBsAg	71.6%	46.7%	31.3%	25.8%	20.4%
iTACT-HBcrAg	65.7%	67.1%	67.8%	64.5%	64.5%
Anti-HBs	31.3%	56%	64.4%	72%	79.6%
HBV DNA	7.2%	0%	0%	0%	0%

Conclusions

- HBcrAg
 - Distinct levels in different phases of chronic hepatitis B disease
 - Pattern follows with other biomarkers: HBsAg/ HBeAg/ HBV DNA/ HBV RNA
 - Detectability/ levels depend on the viral activities
 - Provide alternative/ additional assessment on viral status in natural history of disease, patients on treatment and patients with HBsAg seroclearance
 - Assay sensitivity has been improved from 3 log to 2.1 log U/mL as the lower limit of quantification
 - High detectability rate in patients with HBsAg seroclearance providing continuous assessment after functional cure
 - Other roles include provision of another mean of measurement for target engagement of novel agents with different modes of action and prediction for disease outcome and treatment cessation
 - Continuous refinement and standardization of the assays are required