The 17th NCGM International Infectious Diseases Forum
Sep 5, 2024

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

新たなバイオマーカー高感度HBコア関連抗原測定の臨床的有用性

Takako Inoue[1] and Yasuhito Tanaka[2]

1. Department of Clinical Laboratory Medicine, Nagoya City University Hospital, Nagoya, Japan
2. Department of Gastroenterology and Hepatology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

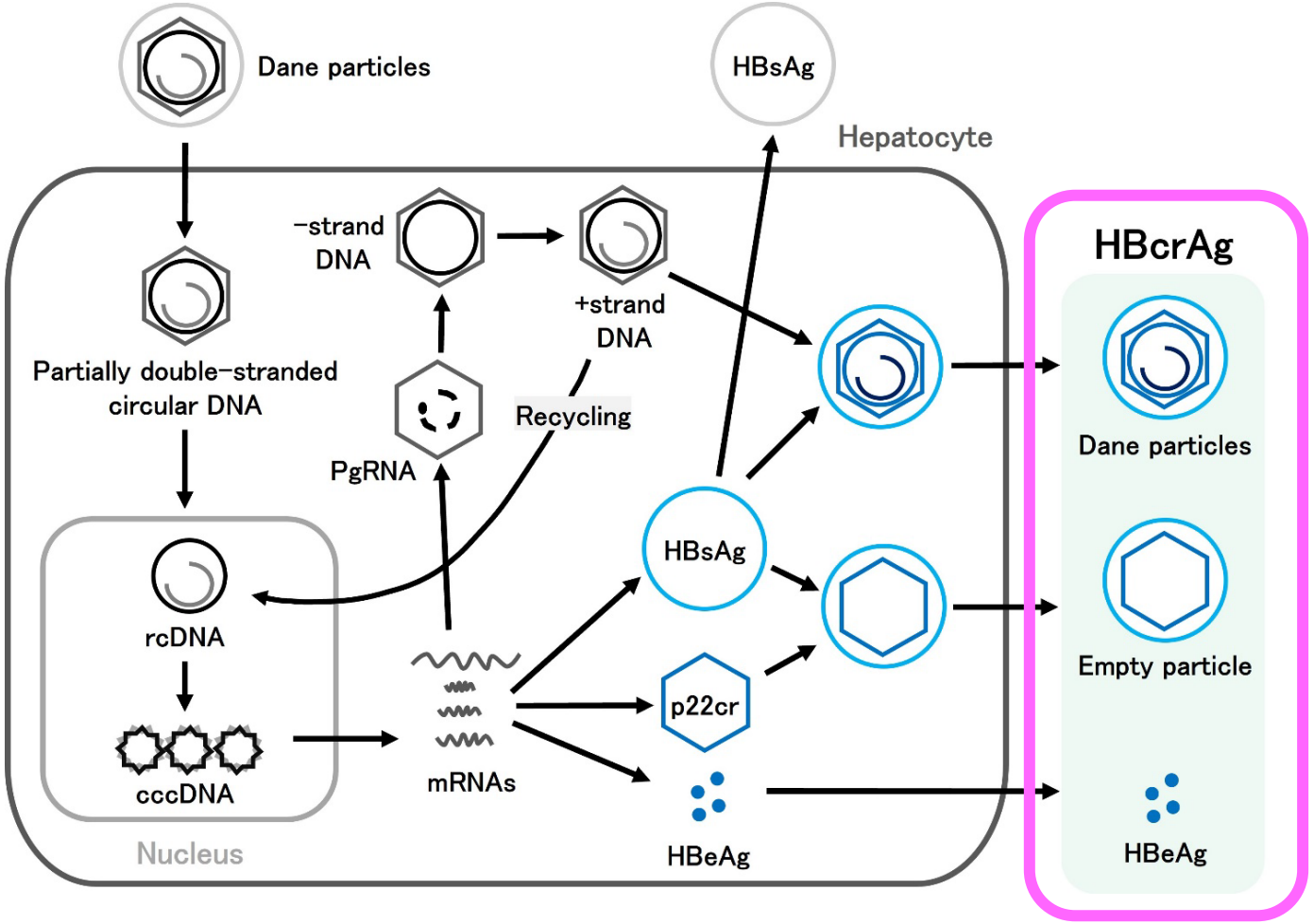
Today's Topics

- Development of a fully automated highly sensitive HBcrAg reagent
  - Characteristics of iTACT-HBcrAg
  - Results of basic performance evaluation
- Utility in the diagnosis of HBV reactivation
- The HBcrAg assay in resource-limited regions

Today's Topics

- Development of a fully automated highly sensitive HBcrAg reagent
  - Characteristics of iTACT-HBcrAg
  - Results of basic performance evaluation
- Utility in the diagnosis of HBV reactivation
- The HBcrAg assay in resource-limited regions

HBV serum markers

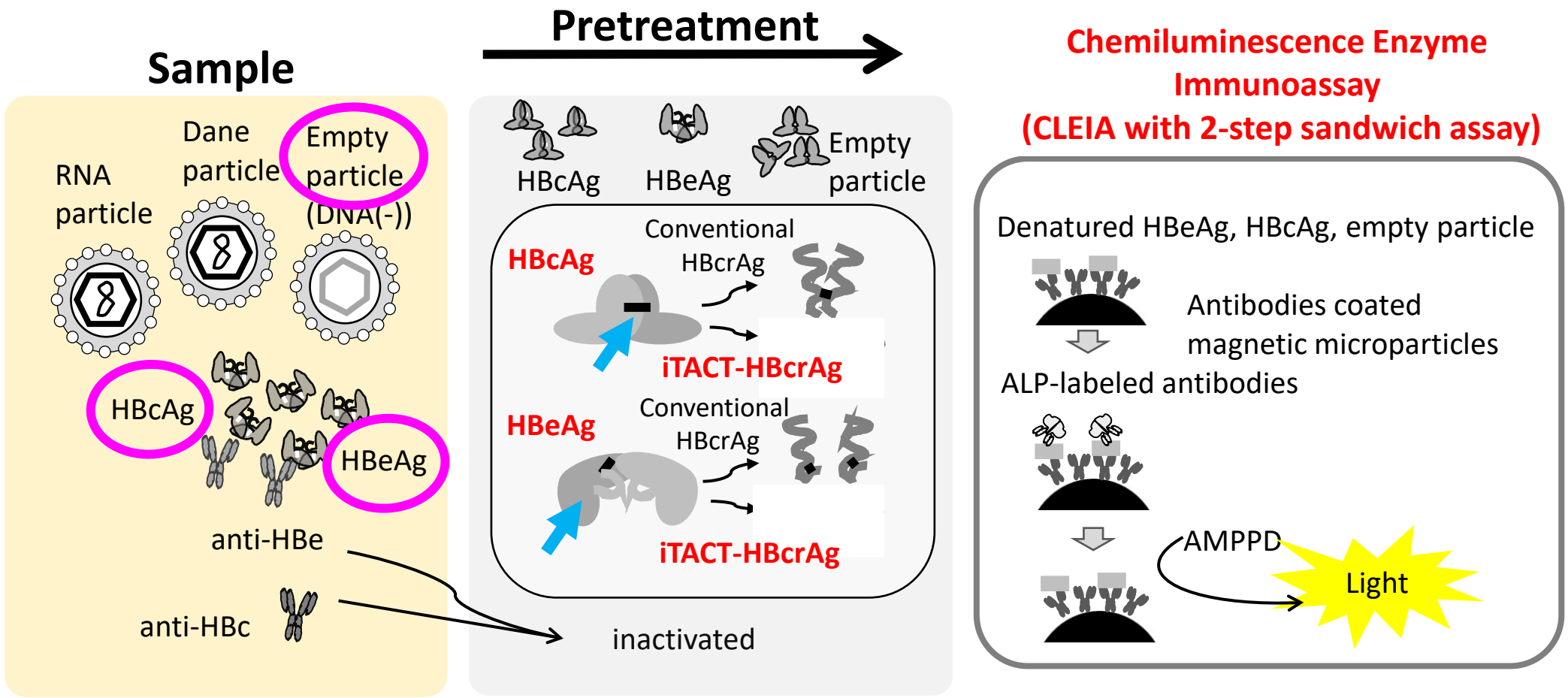


Inoue T and Tanaka Y
Clin Mol Hepatol 2020;26:261-279.

- qHBsAg is produced not only by cccDNA but also integrated HBV DNA.
- HBcrAg reflects the transcriptional activity of intrahepatic cccDNA more accurately than qHBsAg.

iTACT*-HBcrAg assay: Measurement practices

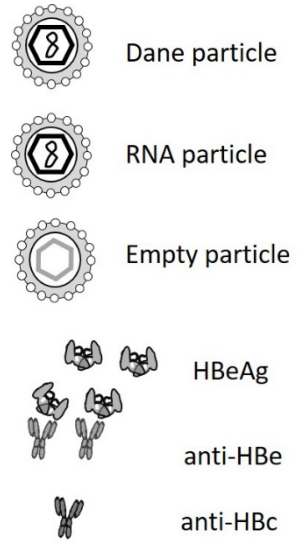
* iTACT: immunoassay for total antigen including complex via pretreatment

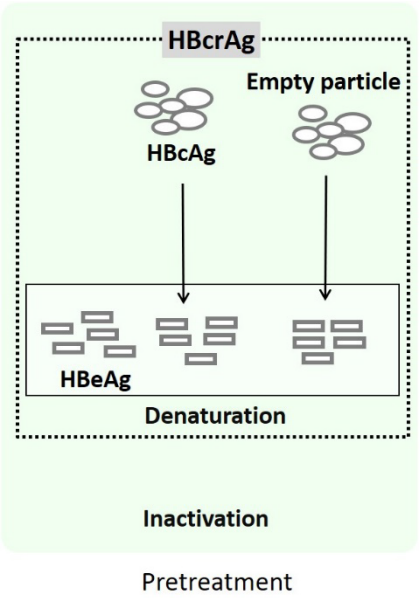


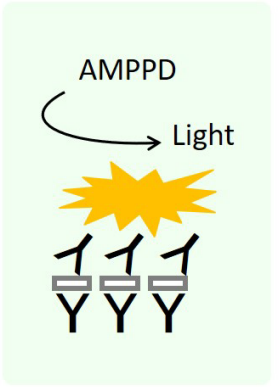
Cleaving the di-sulfide bond with the reducing agent

Inoue, Tanaka et al.
J Hepatol, 2021

Comparison of conventional HBcrAg and iTACT-HBcrAg







CLEIA:
2-step sandwich assay

Inoue and Tanaka. Clin Mol Hepatol, 2023

> 60 minutes

	Serum	Pretreatment	Measurement range
Conventional HBcrAg	150 µL	Manual 60°C for 30 min.	3.0～7.0 log U/mL
iTACT-HBcrAg	**50 µL**	Automatic (on-board) 37°C for **6.5 min**.	**2.1**～7.0 log U/mL

33 minutes

Key points of iTACT-HBcrAg assay

- Fully automated on-board pretreatment

Reduction of measurement time
60 →33 minutes

- Addition of solid-phase antibodies
- Optimization of reagent assay

Achieved higher sensitivity
3.0 →2.1 log U/mL

- Reduction of sample volume required

Reduce burden
150 →50 µL

Today's Topics

- Development of a fully automated highly sensitive HBcrAg reagent
  - Characteristics of iTACT-HBcrAg
  - Results of basic performance evaluation
- Utility in the diagnosis of HBV reactivation
- The HBcrAg assay in resource-limited regions

Report 1

Research Article
Viral Hepatitis

JOURNAL OF HEPATOLOGY

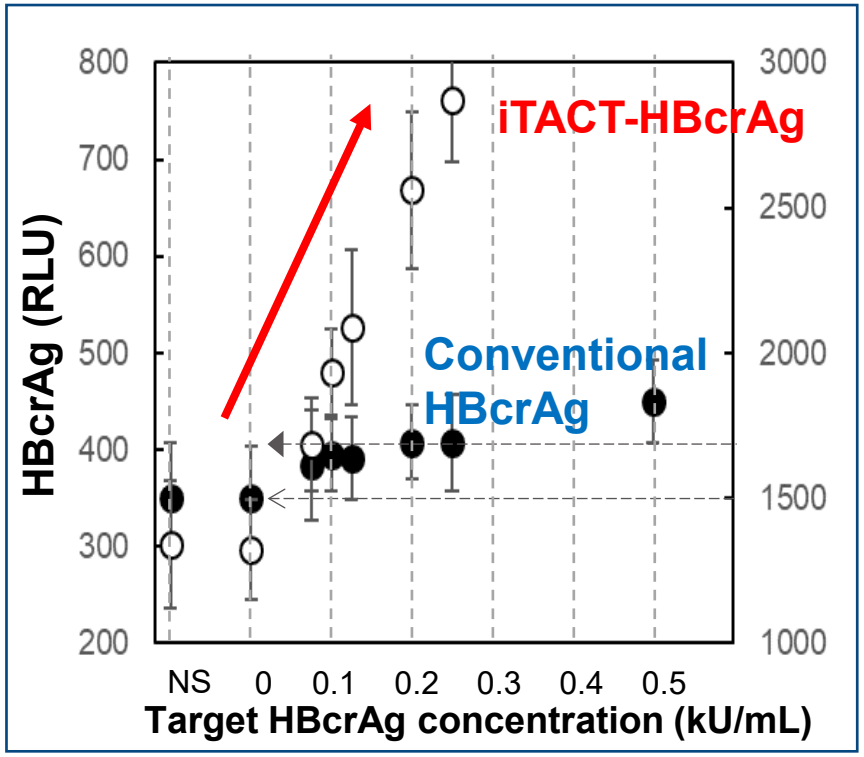
Clinical efficacy of a novel, high-sensitivity HBcrAg assay in the management of chronic hepatitis B and HBV reactivation

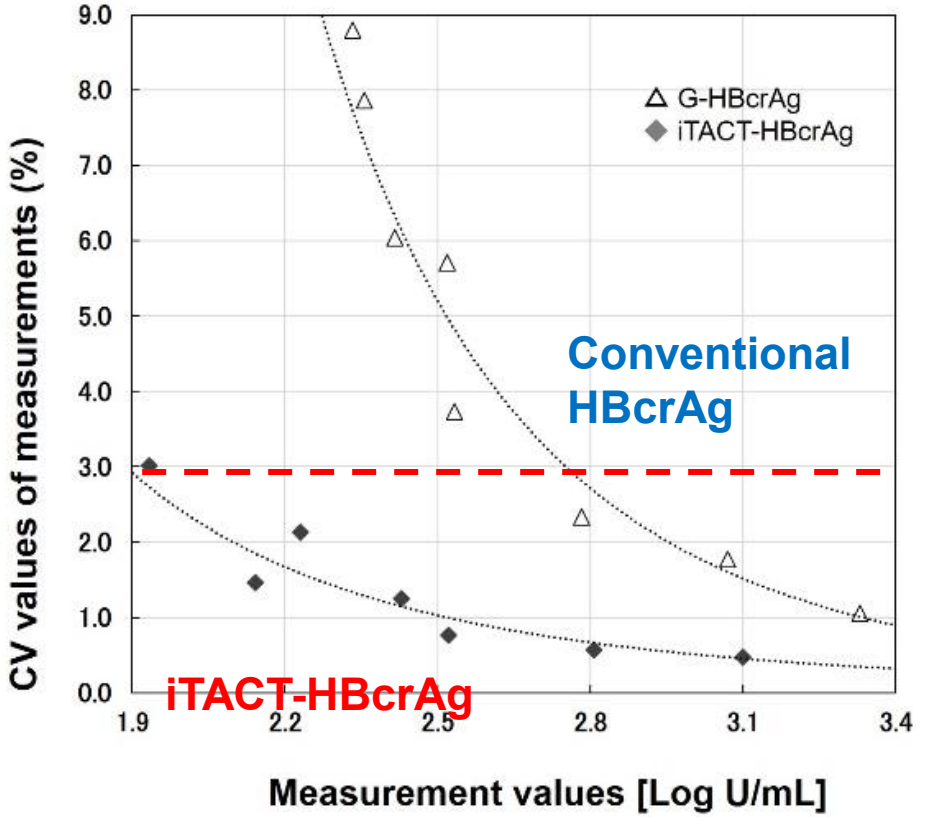
Takako Inoue[1], Shigeru Kusumoto[2], Etsuko Iio[3], Shintaro Ogawa[3], Takanori Suzuki[4], Shintaro Yagi[5], Atsushi Kaneko[6], Kentaro Matsuura[4], Katsumi Aoyagi[5,6], Yasuhito Tanaka[1,3,7,*]

[1]*Department of Clinical Laboratory Medicine, Nagoya City University Hospital, Nagoya, Japan;* [2]*Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan;* [3]*Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan;* [4]*Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan;* [5]*Research and Development Department, Advanced Life Science Institute, Inc., Hachioji, Tokyo, Japan;* [6]*Research and Development Division, Fujirebio Inc., Hachioji, Tokyo, Japan;* [7]*Department of Gastroenterology and Hepatology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan*

- Basic performance evaluation of iTACT-HBcrAg
- Main components of HBcrAg detected in the early phase of HBV reactivation

Assay reliability of iTACT-HBcrAg





The count concentrations below 0.5 kU/mL (2.7 log) were quite different from the negative control in iTACT-HBcrAg.

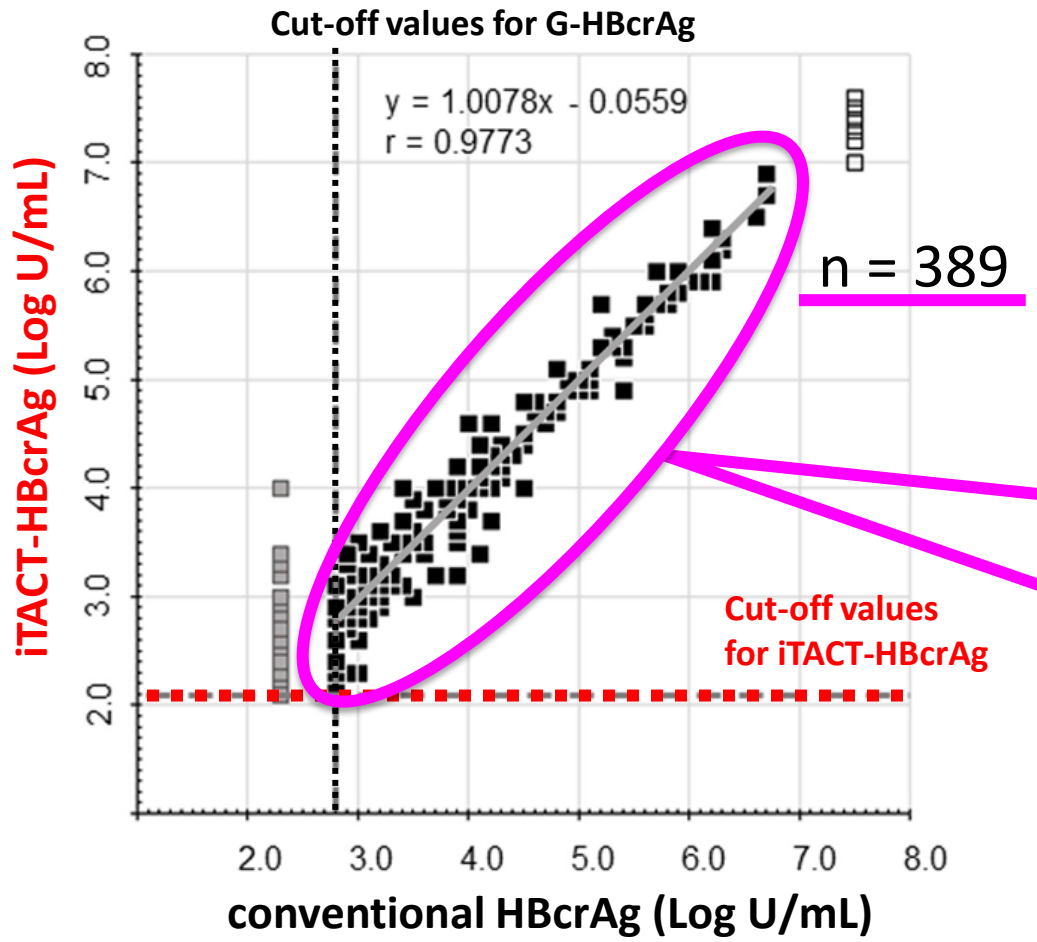
The reliable cut-off values

iTACT-HBcrAg: 2.1 Log U/mL

Conventional HBcrAg: 2.8 Log U/mL

Inoue, Tanaka et al. J Hepatol, 2021

Correlation of HBcrAg levels measured by iTACT-HBcrAg versus conventional HBcrAg (n = 389)



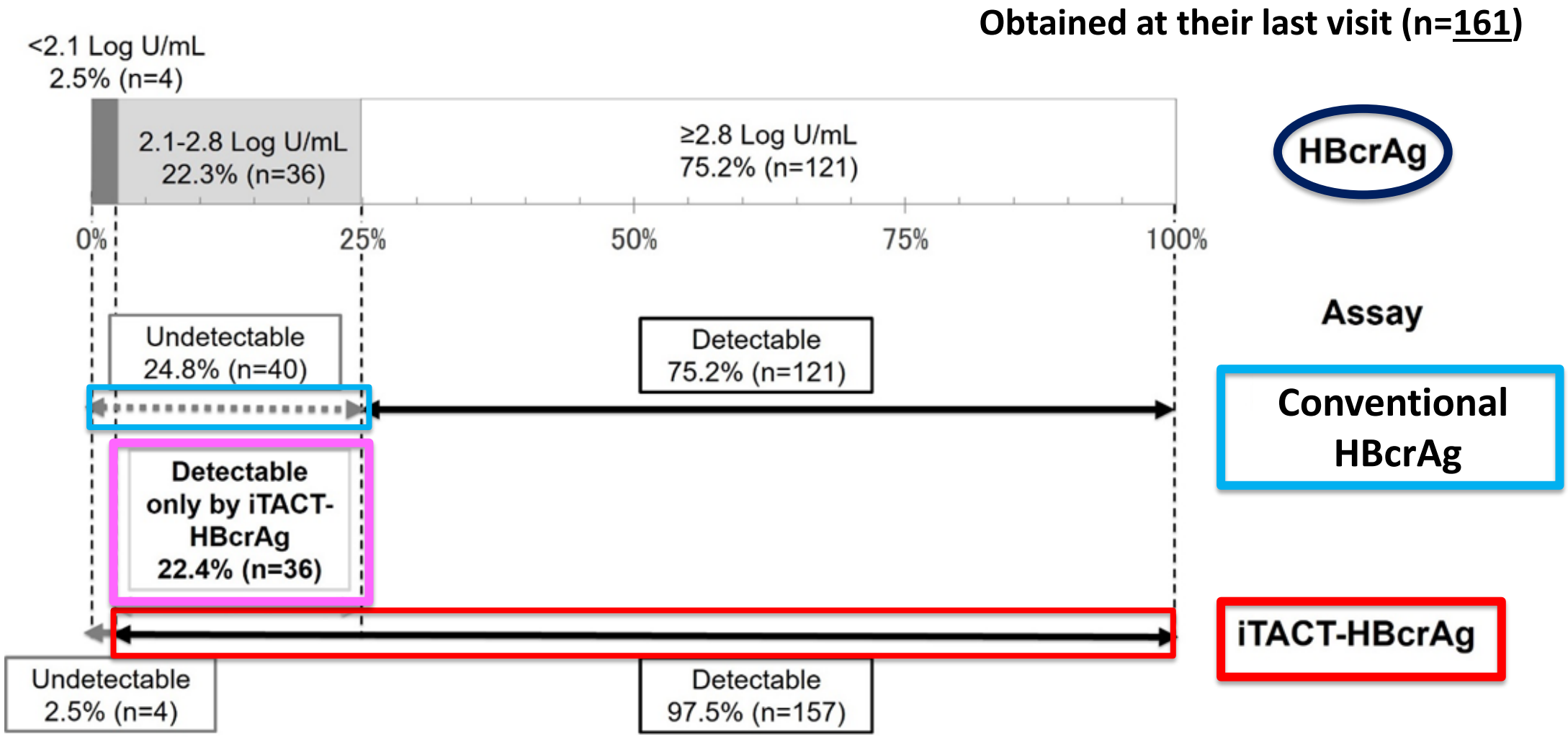
Positive or negative for anti-HBe

The results of the samples found by conventional HBcrAg and iTACT-HBcrAg to have HBcrAg levels of between **≥2.8 Log U/mL and 7 Log U/mL**.

Correlation between conventional assay and iTACT-HBcrAg is good

Inoue, Tanaka et al. J Hepatol, 2021

Comparison between the rates of HBcrAg detection by iTACT-HBcrAg versus conventional HBcrAg in samples from NA-treated patients

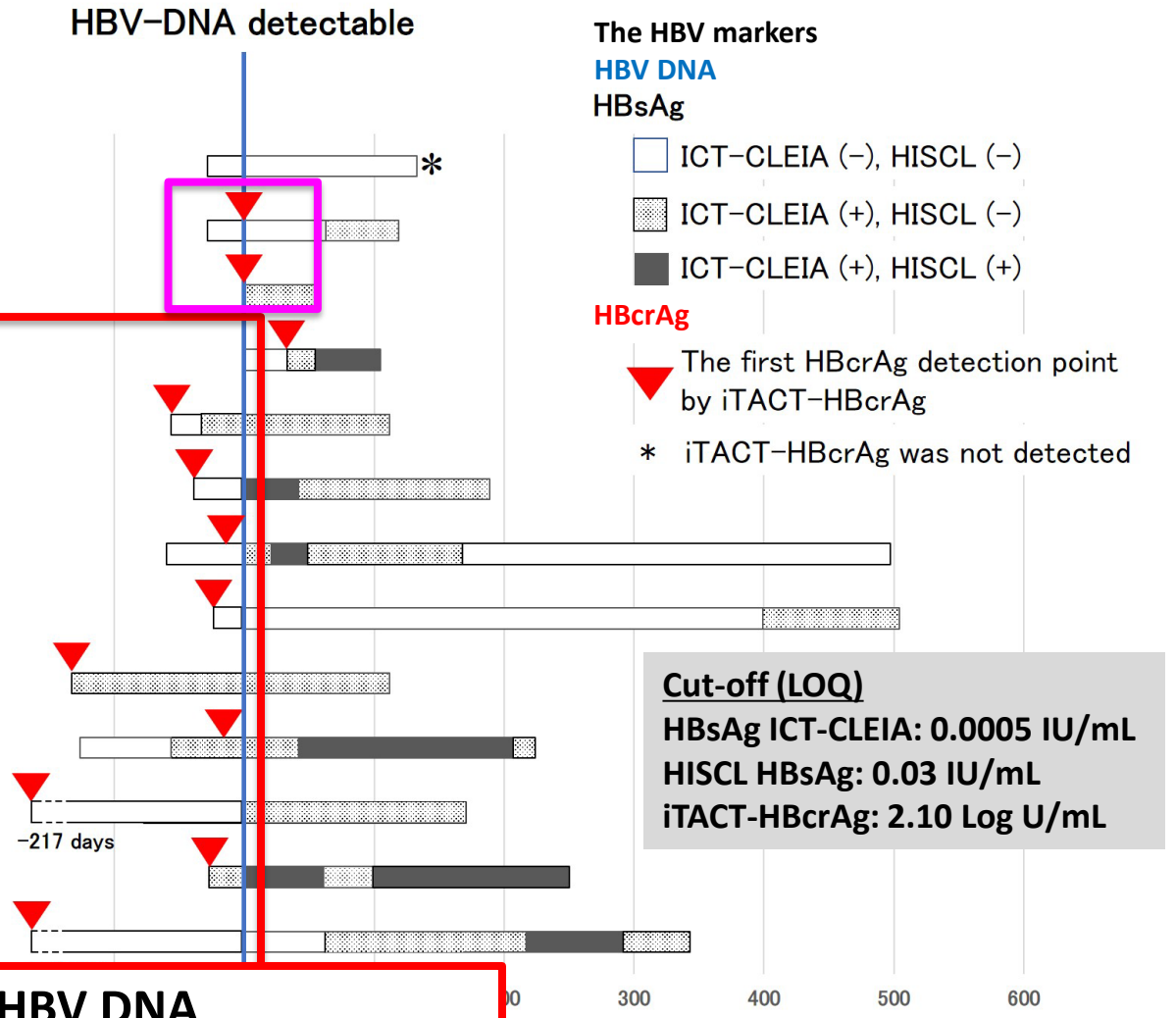


Inoue, Tanaka et al. J Hepatol, 2021

Comparison of assay results of various HBV biomarkers from serial specimens of serum obtained over time from 13 patients who developed HBV reactivation

HBV-reactivated patients

No.	Age	Sex	Diagnosis	Peak HBV DNA (IU/mL)
1	81	M	Multiple myeloma	< 20.0
2	54	M	Malignant lymphoma	28.6
3	81	M	Myelodysplastic syndrome	35.2
4	64	F	Multiple myeloma	44.8
5	82	M	Primary macroglobulinemia	22.9
6	81	M	Idiopathic thrombocytopenic purpura	< 20.0
7	61	M	Multiple myeloma	37.1
8	83	F	Multiple myeloma	29.5
9	70	M	Metastatic bone sarcoma	< 20.0
10	58	F	Adult T-cell leukemia/lymphoma	29.5
11	58	M	Malignant lymphoma	32.4
12	65	F	Adult T-cell leukemia/lymphoma	21.9
13	76	M	Rheumatoid arthritis	41.0



Cut-off (LOQ)
HBsAg ICT-CLEIA: 0.0005 IU/mL
HISCL HBsAg: 0.03 IU/mL
iTACT-HBcrAg: 2.10 Log U/mL

Comparison between HBcrAg and HBV DNA

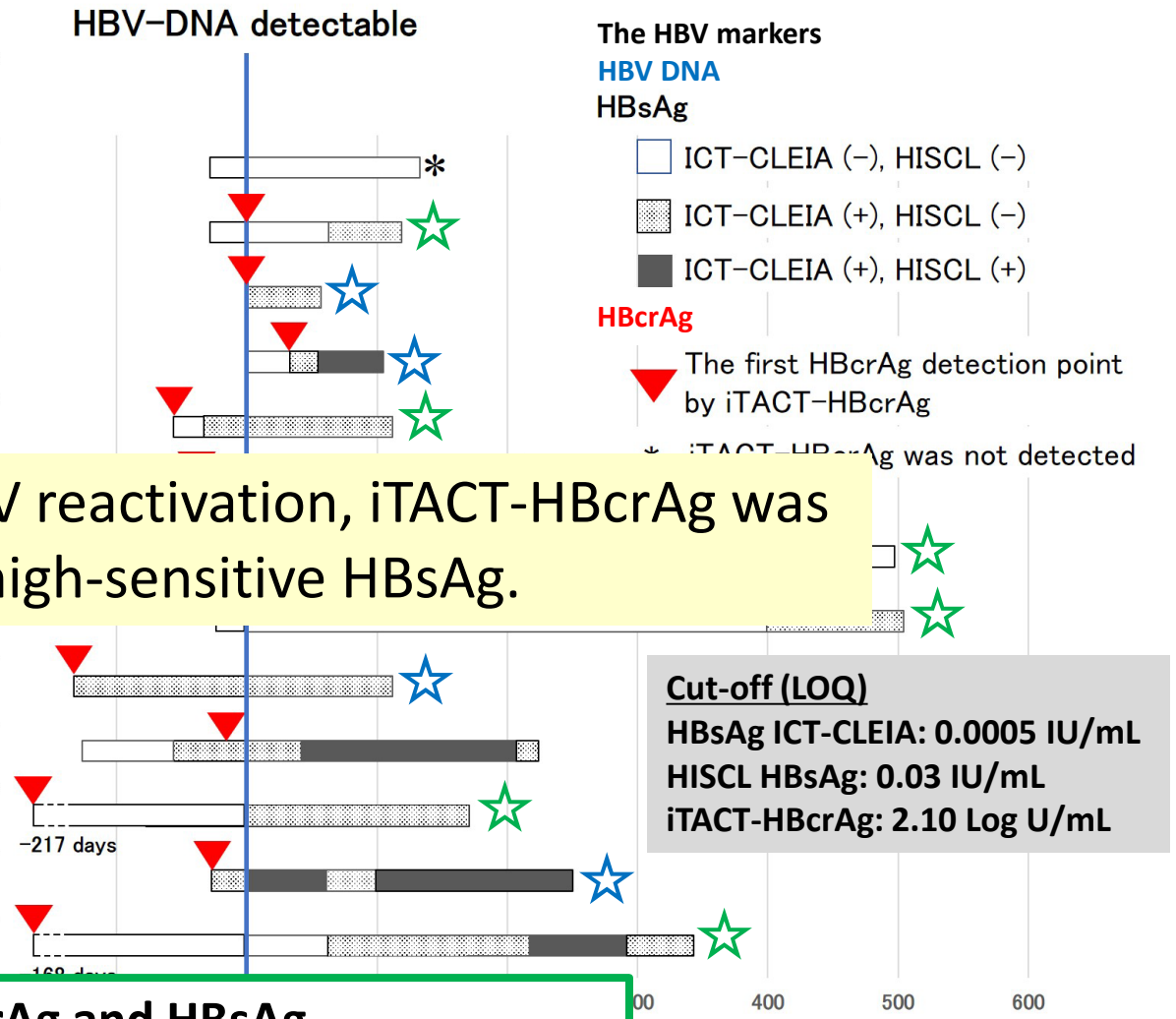
Nine and **2** of 13 HBV-reactivated patients were HBcrAg-positive by iTACT-HBcrAg **before** and **at** HBV DNA positivity, respectively.

oue, Tanaka et al. J Hepatol, 2021

Comparison of assay results of various HBV biomarkers from serial specimens of serum obtained over time from 13 patients who developed HBV reactivation

HBV-reactivated patients

No.	Age	Sex	Diagnosis	Peak HBV DNA (IU/mL)
1	81	M	Multiple myeloma	< 20.0
2	54	M	Malignant lymphoma	28.6
3	81	M	Myelodysplastic syndrome	35.2
4	64	F	Multiple myeloma	44.8
5	82	M	Primary macroglobulinemia	22.9
6				
7				
8	83	F	Multiple myeloma	29.5
9	70	M	Metastatic bone sarcoma	< 20.0
10	58	F	Adult T-cell leukemia/lymphoma	29.5
11	58	M	Malignant lymphoma	32.4
12	65	F	Adult T-cell leukemia/lymphoma	21.9
13	76	M	Rheumatoid arthritis	41.0



Regarding the patients with HBV reactivation, iTACT-HBcrAg was clearly detectable earlier than high-sensitive HBsAg.

Comparison between HBcrAg and HBsAg

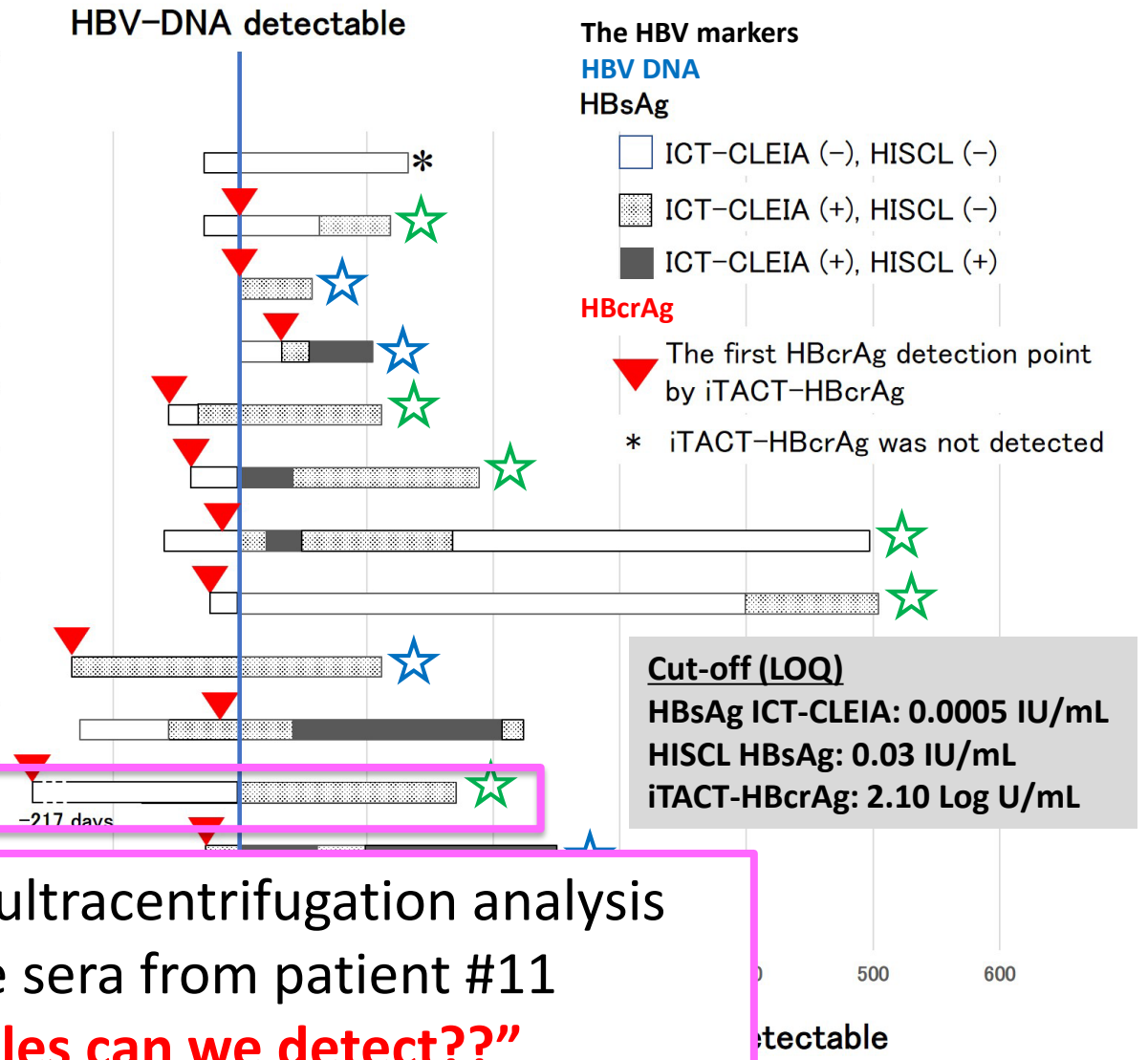
Seven and **4** patients were HBcrAg-positive by iTACT-HBcrAg **before** and **at** being HBsAg-positive by ICT-CLEIA, respectively.

naka et al. J Hepatol, 2021

Comparison of assay results of various HBV biomarkers from serial specimens of serum obtained over time from 13 patients who developed HBV reactivation

HBV-reactivated patients

No.	Age	Sex	Diagnosis	Peak HBV DNA (IU/mL)
1	81	M	Multiple myeloma	< 20.0
2	54	M	Malignant lymphoma	28.6
3	81	M	Myelodysplastic syndrome	35.2
4	64	F	Multiple myeloma	44.8
5	82	M	Primary macroglobulinemia	22.9
6	81	M	Idiopathic thrombocytopenic purpura	< 20.0
7	61	M	Multiple myeloma	37.1
8	83	F	Multiple myeloma	29.5
9	70	M	Metastatic bone sarcoma	< 20.0
10	58	F	Adult T-cell leukemia/lymphoma	29.5
11	58	M	Malignant lymphoma	32.4
12	65			
13	76			



OptiPrep density gradient ultracentrifugation analysis was performed for the sera from patient #11

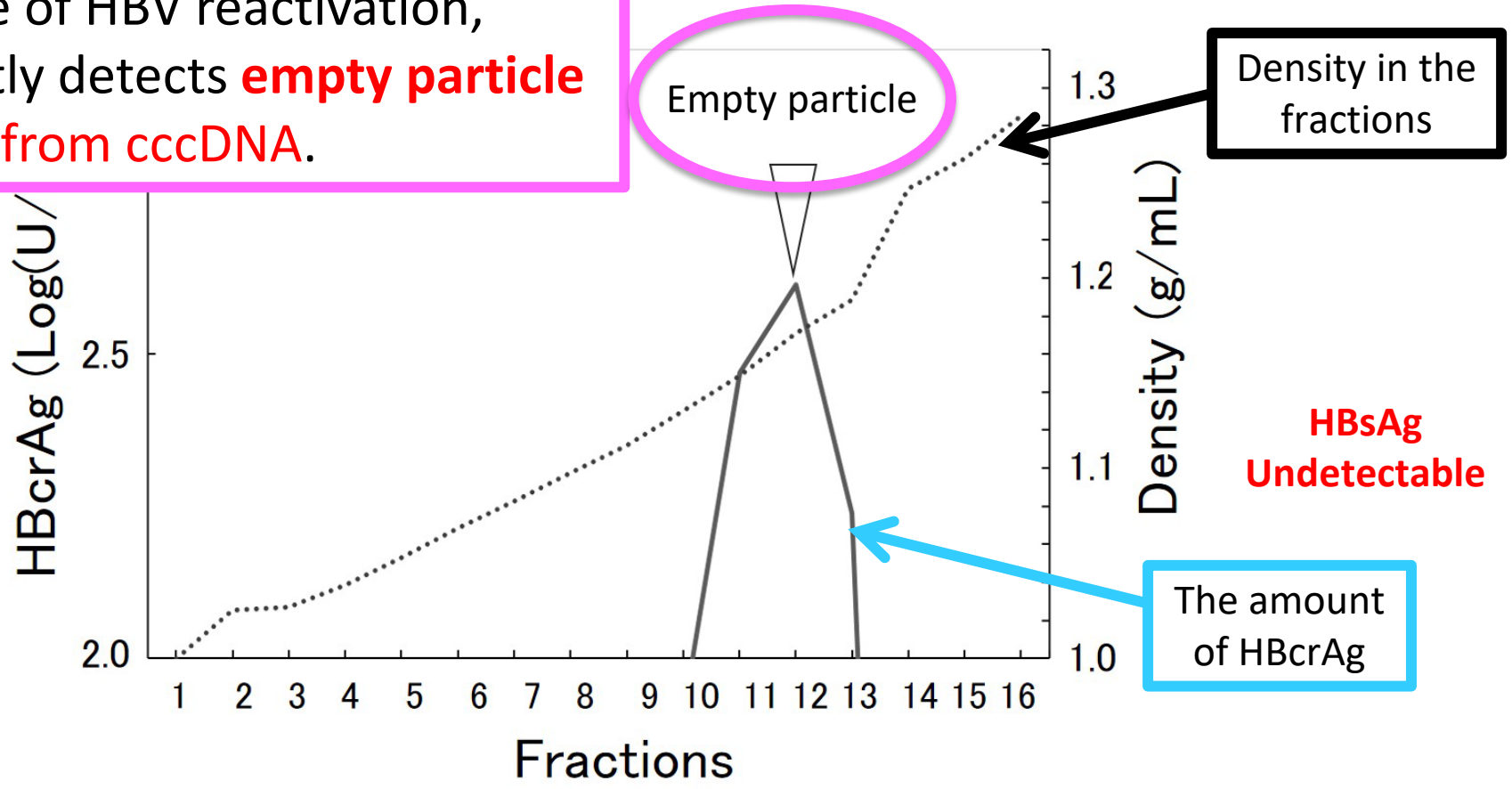
"What kind of particles can we detect??"

Inoue, Tanaka et al. J Hepatol, 2021

OptiPrep density gradient ultracentrifugation analysis of pre-HBV DNA detection in a patient with HBV reactivation

From case #11 taken 133 days before HBV DNA was detected

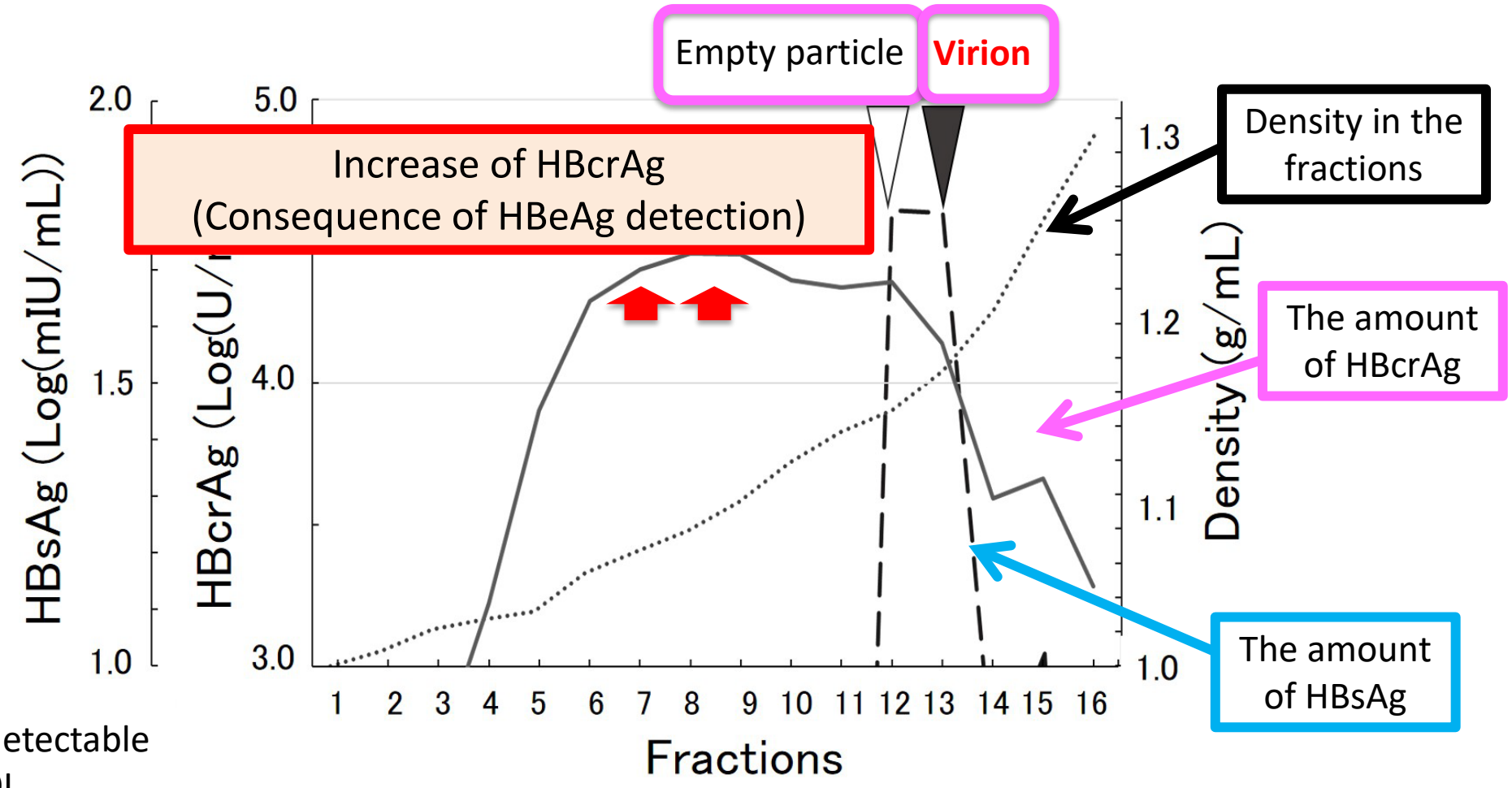
In the early phase of HBV reactivation, HBcrAg dominantly detects **empty particle** produced mainly from cccDNA.



Inoue, Tanaka et al. J Hepatol, 2021

OptiPrep density gradient ultracentrifugation analysis of post-HBV DNA detection in a patient with HBV reactivation

From case #11 taken 49 days after HBV DNA was detected



HBV DNA; undetectable
HBeAg; 2.6 COI
HBsAg; 0.0135 IU/mL (ICT-CLEIA)

Inoue, Tanaka et al. J Hepatol, 2021

Today's Topics

- Development of a fully automated highly sensitive HBcrAg reagent
  - Characteristics of iTACT-HBcrAg
  - Results of basic performance evaluation

- Utility in the diagnosis of HBV reactivation

- The HBcrAg assay in resource-limited regions

Report 2

Received: 16 January 2022 | Revised: 13 February 2022 | Accepted: 18 February 2022

DOI: 10.1111/hepr.13761

ORIGINAL ARTICLE

Hepatology Research

Management of hepatitis B virus (HBV) reactivation in patients with resolved HBV infection based on a highly sensitive HB core-related antigen assay

Shinya Hagiwara[1] | Shigeru Kusumoto[1] | Takako Inoue[2] | Shintaro Ogawa[3] | Tomoko Narita[1] | Asahi Ito[1] | Masaki Ri[1] | Hirokazu Komatsu[1] | Takanori Suzuki[4] | Kentaro Matsuura[4] | Shintaro Yagi[5] | Atsushi Kaneko[6] | Katsumi Aoyagi[5,6] | Shinsuke Iida[1] | Yasuhito Tanaka[3,7]

- HBV reactivation patients with hematopoietic malignancies (n=27)
- Comparison of detection rates by high-sensitivity HBcrAg and high-sensitivity HBsAg

Report 3

J Gastroenterol (2022) 57:486–494
https://doi.org/10.1007/s00535-022-01872-w

The Japanese Society of Gastroenterology

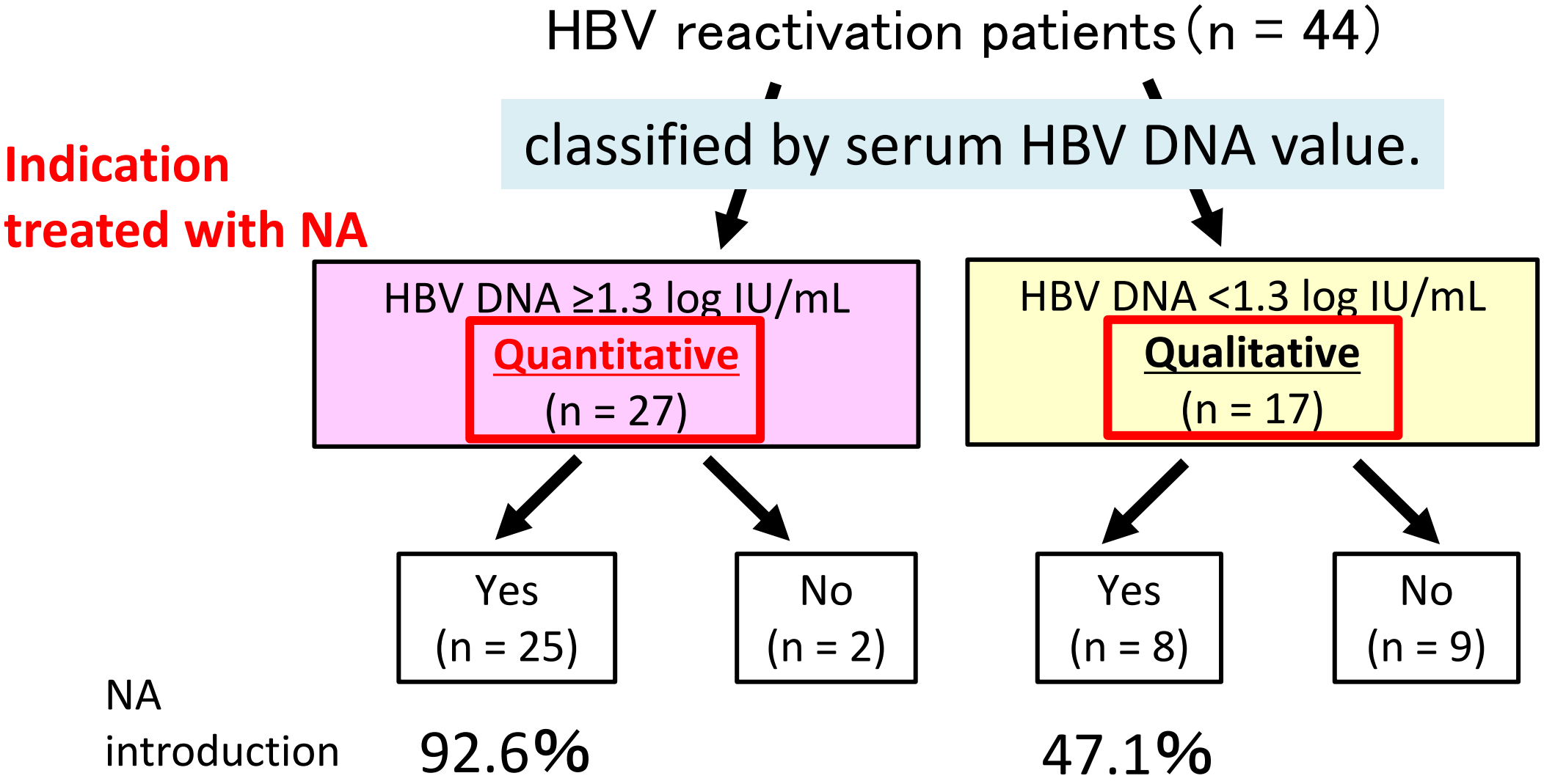
ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clinical usefulness of a novel high-sensitivity hepatitis B core-related antigen assay to determine the initiation of treatment for HBV reactivation

Takanori Suzuki[1] · Takako Inoue[1] · Kentaro Matsuura[1] · Shigeru Kusumoto[1] · Shinya Hagiwara[1] · Shintaro Ogawa[1] · Shintaro Yagi[2] · Atsushi Kaneko[3] · Kei Fujiwara[1] · Takehisa Watanabe[4] · Katsumi Aoyagi[2,3] · Yukitomo Urata[5] · Akihiro Tamori[6] · Hiromi Kataoka[1] · Yasuhito Tanaka[1,4]

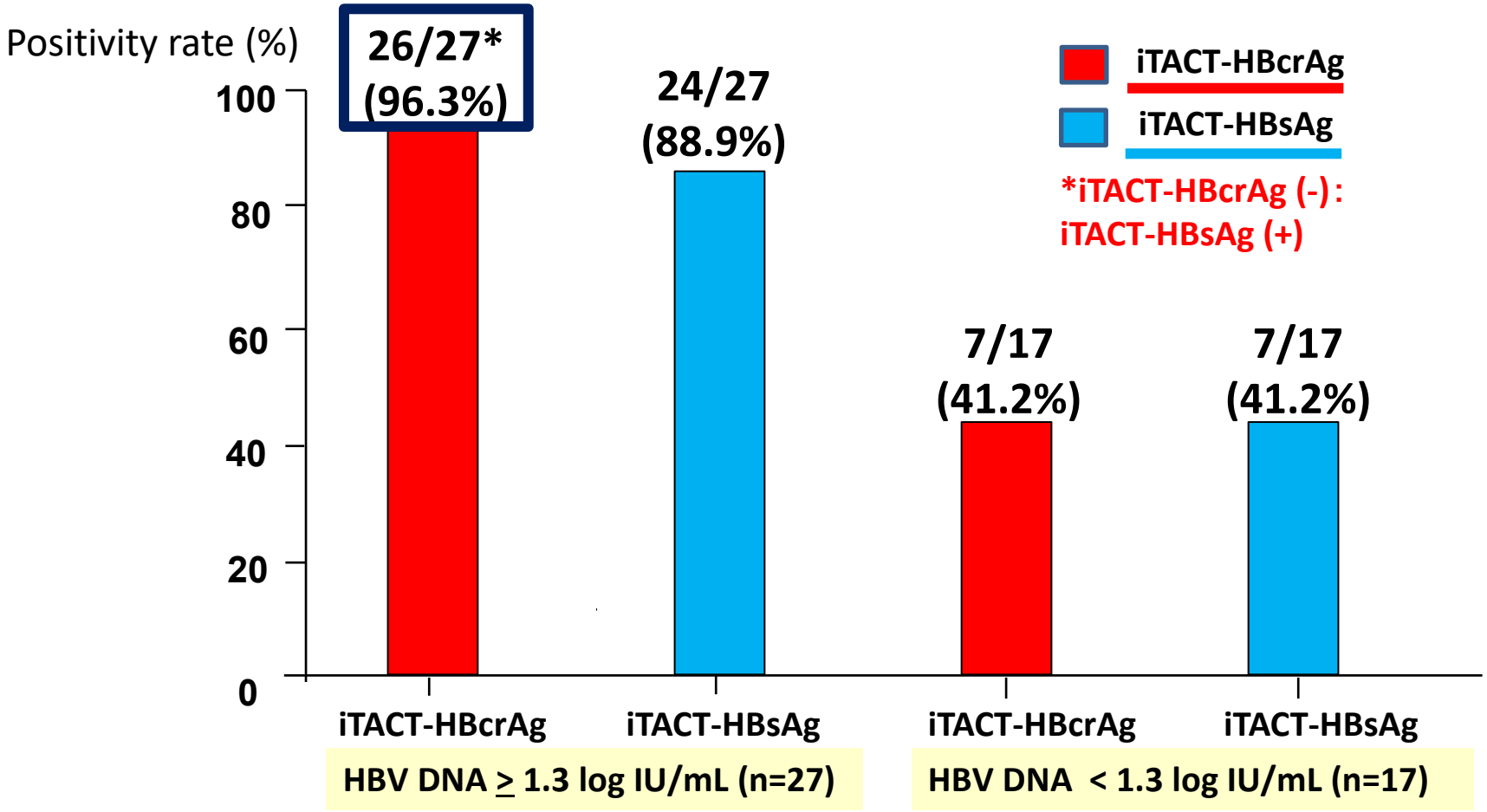
- HBV reactivation patients（n = 44）
- Comparison of detection rates by iTACT-HBcrAg and HBV DNA quantification

Patients and methods



Suzuki T, Inoue T and Tanaka Y, et al. J Gastroenterol, 2022

Comparison of positivity of iTACT-HBsAg and iTACT-HBcrAg



Positivity for iTACT-HBsAg and iTACT-HBcrAg:
Potential as an indicator of NA treatment introduction

Suzuki T, Inoue T and Tanaka Y, et al. J Gastroenterol, 2022

Today's Topics

- Development of a fully automated highly sensitive HBcrAg reagent
  - Characteristics of iTACT-HBcrAg
  - Results of basic performance evaluation
- Utility in the diagnosis of HBV reactivation
- The HBcrAg assay in resource-limited regions

Who to treat among people with CHB from WHO

TDF antiviral prophylaxis (from at least the second trimester of pregnancy until at least delivery or completion of the infant HBV vaccination series)

- **If there is access to HBV DNA or HBeAg serology:** TDF prophylaxis for HBsAg-positive pregnant women with HBV DNA ≥200 000 IU/mL or positive HBeAg
- **If there is no access to HBV DNA or HBeAg serology:** TDF prophylaxis for all HBsAg-positive pregnant women.

7.3.2 New recommendation – TDF prophylaxis for all HBsAg-positive pregnant women

Since 2020, WHO has recommended that HBsAg-positive pregnant women at high risk of transmitting HBV to their infants because of high HBV DNA (≥200 000 IU/mL) or positive HBeAg receive peripartum antiviral prophylaxis using TDF, preferably from the 28th week of pregnancy until at least delivery to prevent MTCT of HBV *(3)*. This recommendation is in addition to providing HepB3 for all infants (starting with a timely hepatitis B birth dose). However, significant challenges remain in accessing HBV DNA viral load or HBeAg serology testing among HBsAg-positive pregnant women to determine eligibility for antiviral prophylaxis, especially in sub-Saharan Africa.

To date, no studies have been undertaken to examine the clinical impact and feasibility of expanding antiviral prophylaxis access to all HBsAg-positive pregnant women. Therefore, WHO commissioned a modelling study of different scenarios of eligibility for antiviral prophylaxis.

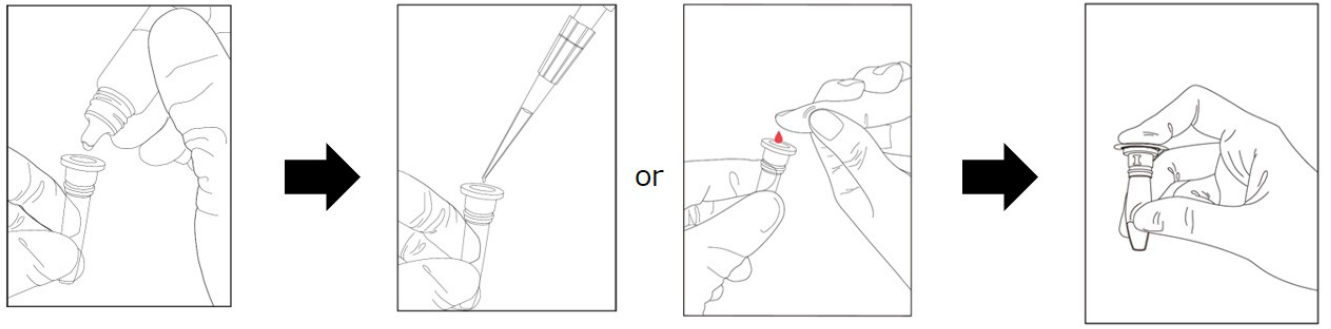
World Health Organization

Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection
March 2024

HBcrAg detection procedure by HBcrAg-RDT

HBcrAg-RDT
(Rapid diagnostic test)

Procedure
(serum, plasma, whole blood)



1. Add Pretreatment solution

2. Add sample

3. Put on the seal and squeeze for 10 seconds or 30 times, then leave for 10min

4. Add neutralizing solution

5. Put on the seal and squeeze for 10 seconds or 30 times

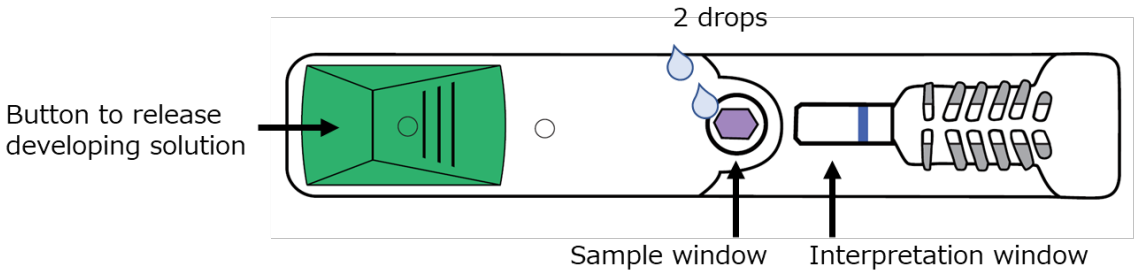
6. Insert the applicator tip onto the top of tube and apply 2 drops

7. Press the convex button of the Reaction Cassette down immediately after applying the sample to start the reaction.

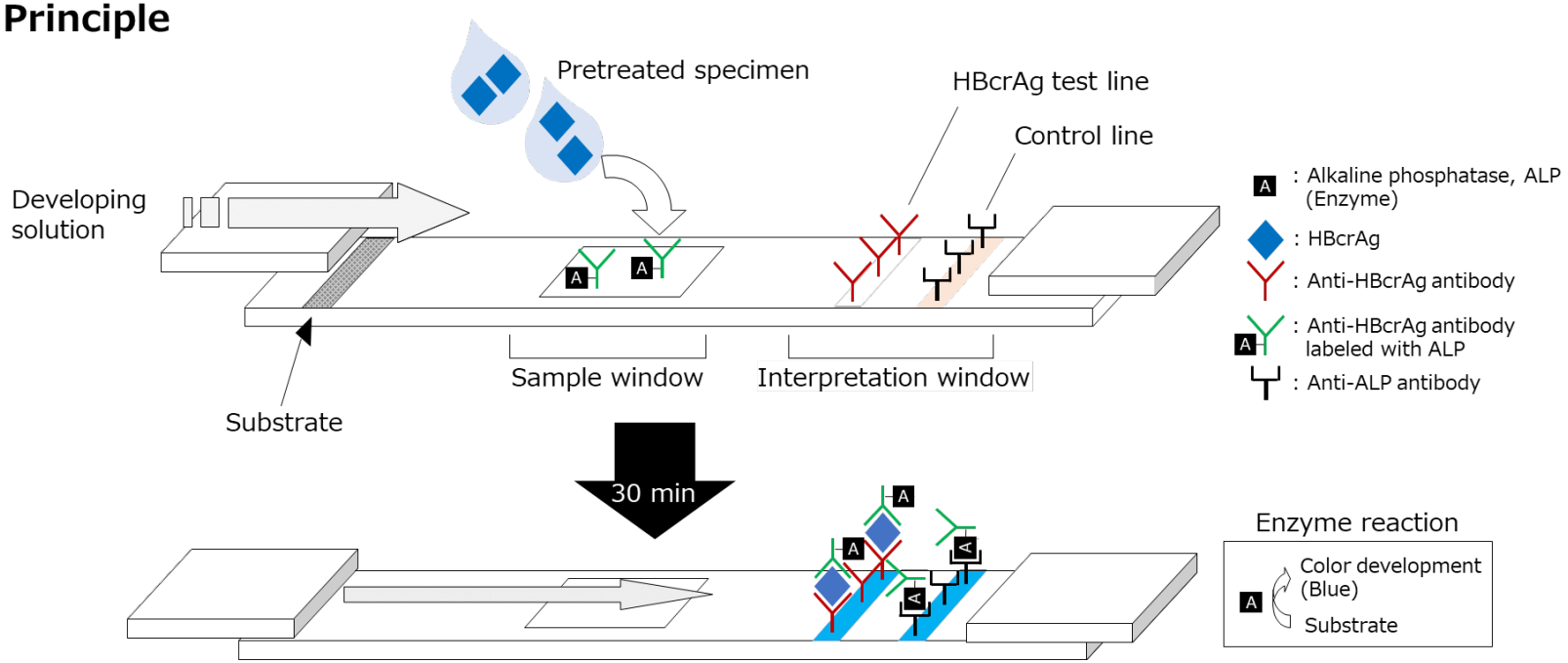
Immunochromatography kits are allowed to stand for 30 minutes before being judged.

Hepatitis B core-related antigen by rapid diagnostic test

Device



Principle



Immunochromatography

Introduction of HBcrAg-RDT in Africa and Asia

Performance of HBcrAg-RDT to diagnose high HBV DNA levels ≥ 200,000 IU/mL

Country	Population	Sample type	N	Sensitivity	Specificity
The Gambia	Adults	Stored sera	284	91%	86%
Cambodia	Pregnant women	Stored sera	1194	94%	95%
Cameroon	Pregnant women	Stored sera	502	91%	93%
Burkina Faso	Postpartum mothers	Capillary blood	154	83%	95%

Shimakawa Y et al., Clin Gastroenterol Hepatol 2022
Vincent JP et al., Global Hepatitis Summit 2023

Summary

Characteristics of iTACT-HBcrAg

- Fully automated and quick
  - Suitable for preclinical examination
- 10-times higher sensitivity (≥ 2.1 logU/mL)

For HBV reactivation

- iTACT-HBcrAg was detectable earlier and longer than HBV DNA and high-sensitive HBsAg.

For screening in resource-limited regions

- Quick and enough sensitivity
- Detection of all persons to be treated

Acknowledgement

- **Dr. Shintaro Yagi**

Research and Development Department, Advanced Life Science Institute, Inc.

- **Drs. Atsushi Kaneko and Yoshiyuki Kitamura**

Research and Development Division, Fujirebio Inc.

- **Dr. Katsumi Aoyagi**

Research and Development Department, Advanced Life Science Institute, Inc.

Research and Development Division, Fujirebio Inc.

- **Dr. Shigeru Kusumoto**

Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences

- **Drs. Etsuko Iio and Shintaro Ogawa**

Department of Gastroenterology and Hepatology, Faculty of Life Sciences, Kumamoto University

- **Drs. Takanori Suzuki and Kentaro Matsuura**

Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences