This guidance was written prior to the February 27, 1997 implementation of FDA's Good Guidance Practices, GGP's. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP's.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

Date

From Chief, Microbiology Branch, Division of Clinical Laboratory Devices, Office of Device Evaluation, Center for Devices and Radiological Health (HFZ-440)

Subject Review Criteria for Devices Intended for the Detection of Hepatitis B "e" Antigen and Antibody to HBe

To Interested Parties

We have developed a draft document entitled "Review Criteria for Assessment of Hepatitis B "e" Antigen and Antibody to Hepatitis B "e" Antigen ". Since the document lists items we will be reviewing, it is intended to assist manufacturerin the preparation of premarket approval submissions for these types of devices.

We are soliciting your ideas, recommendations, and comments regarding the enclosed review criteria. We will appreciate receiving your comments so that we can incorporate as many suggestions as possible in a revision. Additional copies of this document may be obtained through the Division of Small Manufacturers Assistance from Geoffrey Clark, at (301) 443-6597.

Please address comments to:

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Enclosure

Original Version
December, 1991

REVIEW CRITERIA FOR ASSESSMENT OF HEPATITIS B "e" ANTIGEN AND ANTIBODY TO HEPATITIS B "e" ANTIGEN IN VITRO DIAGNOSTIC DEVICES

This is a flexible document representing the current major concerns and issues regarding hepatitis B "e" antigen (HBeAg) and antibody to hepatitis B "e" antigen (Anti-HBe), *in vitro* diagnostic devices. It is based on 1) current basic science, 2) clinical experience, 3) previous premarket approval application (PMA) submissions by manufacturers to the Food and Drug Administration (FDA), and 4) the Safe Medical Devices Act (SMDA) of 1990 and FDA regulations in the Code of Federal Regulations (CFR). As advances are made in science and medicine, these review criteria will be re-evaluated and revised as necessary.

PURPOSE OF THE GUIDANCE DRAFT:

This document is an adjunct to 21 CFR Parts 800 - 1299. While it is not to supersede the CFR, this document provides additional guidance and clarification on what information is necessary before the FDA can approve a device for marketing. The FDA can make more informed decisions based on a uniform data base.

DEFINITION OF DEVICE:

The document discusses all generic type devices intended for use in clinical laboratories for the detection of HBeAg and Anti-HBe in human serum or plasma as an aid in the diagnosis and monitoring of patients with hepatitis B virus (HBV).

PRODUCT CODE: LOM

CLASSIFICATION: Class III

PANEL: Microbiology

REVIEW REQUIRED: PREMARKET APPROVAL APPLICATION

1. CLINICAL INDICATION/SIGNIFICANCE/INTENDED USE

Viral hepatitis is a common and serious infectious disease caused by several viral agents and marked by necrosis and inflamation of the liver.[1] At present five hepatitis virus agents have been identified: hepatitis A virus (HAV), HBV, hepatitis C virus (HCV), hepatitis D virus (Delta hepatitis)(HDV), and hepatitis E virus (HEV) (enterically transmitted non-A, non-B hepatitis). Although HBV appears to be a more serious disease than HAV, the initial clinical manifestations of acute viral hepatitis are nonspecific, and it is usually not possible to reliably differentiate hepatitis type without specific serological markers.[1,2]

Devices have been developed for most of the viral hepatitis agents, however, not all are regulated by the Center for Devices and Radiological Health (CDRH). The hepatitis assays used as diagnostic tests are regulated by CDRH. The hepatitis assays used to screen blood donors are regulated by the Center for Biologics Evaluation and Research (CBER). For example:

REVIEWED BY CDRH	REVIEWED BY CBER
Anti-HAV, Total	HBsAg
Anti-HAV, IgM	Anti-HBs
HBeAg	Anti-HBc
Anti-HBe	HBcAg
Anti-Delta	HCVAg

HBV, originally designated the Dane particle, is an antigenically complex virus containing several serological markers, e.g., HBsAg (originally designated Australia or Au Ag and hepatitis-associated antigen), HBcAg, and hepatitis B e antigen (HBeAg). HBV infection is spread predominantly by the parenteral route.[3] It often occurs in persons with exposure to blood or blood products (multiply transfused patients, hemophiliacs, renal dialysis and oncology patients), exposure to contaminated needles and syringes (medical personnel, drug addicts), persons with multiple sexual contacts, and with exposure to other potentially infectious biological waste.

HBsAg appears in the serum during the incubation period 2-7 weeks before the onset of symptoms, persists during the acute phase, and disappears during convalescence.[1] Anti-HBc Total and IgM appears at the onset of symptoms; however, the anti-HBc IgM disappears 6-24 months after the illness. HBeAg is found only in blood of HBsAg-positive persons, with rare exceptions, during acute HBV infection. It appears approximately 1 week after symptoms appear and usually disappears after several weeks but may persist in chronic carriers of HbsAg. The presence of HBeAg in the serum correlates with active HBV replication and

relatively high infectivity, thus it is considered to be an early marker of HBV infection.[2,3] The disappearance of HBeAg and the appearance of antibody to HBeAg (anti-HBe) in HBsAg-positive persons indicates "seroconversion" which corresponds to a relative decrease in HBV replication and infectivity. Persistence of HBeAg and failure to seroconvert beyond 8-10 weeks implies a chronic carrier state of HBV and persistent high infectivity and/or ongoing disease.[1,4]

2. DEVICE DESCRIPTION

The device is usualy marketed as a combined kit to determine both viral antigen and viral antibodies :

a) HBeAg: for the qualitative (only) determination of HBe antigen in human serum or plasma of persons with acute or chronic HBV infection.

b) Anti-HBe: for the qualitative determination of total antibody directed against HBe antigen in human serum or plasma and as an aid in the diagnosis of or monitoring of patients with hepatitis B.

Each manufacturer must discuss in the Summary of Safety and Effectiveness Data (SS&ED) section and in the package insert:

a) The intended use and indications for use of the device. Each device will have the same intended use as the generic device (see definition).

b) The principles of the device methodology with a brief history of the specific technology/methodology upon which the device is based. Technology employed in previous submissions includes radioimmunoassay (RIA), enzyme linked immunoassay (ELISA), and the use of monoclonal antibodies.

   New technologies that may raise new issues of safety and effectiveness are not the subject of this draft document. They include but are not limited to; deoxyribonucleic acid (DNA) probes, polymerase chain reaction (PCR), or any other nucleic acid amplification technique.

c) Interpretive criteria based on assay type, i.e., semi-quantiative (ability to titer single sample type) or qualitative (serological status - single serum sample) with a procedure for calculating cutoff value where appropriate.

3. SPECIFIC PERFORMANCE CONSIDERATIONS:

A device for which an FDA approved PMA exists should be established as a reference method and assayed in parallel with the new device in all studies. Once established, the reference method must not be changed during any phase of the study. Comprehensive study protocols must be included in the PMA submission.

All assays must be performed with the final production form of the device, not a prototype, following directions provided in the package insert. For devices that have alternative procedures, e.g., overnight vs. shortened incubation, or claim to be suitable to be used with both serum and plasma samples, studies must be performed using both procedures and sample types. The manufacturer should also inform users of advantages or disadvantages of both procedures, e.g., any loss of sensitivity, etc..

Other guidance for presentation of data can be obtained from the SS&ED section of a recently approved PMA.

A. PRE-CLINICAL STUDIES:

Pre-clinical studies are performed in the manufacturer's facility or at a designated site in order to assess specific performance of the device.

Studies done in the pre-clinical trials should include:

1) Analytical Sensitivity - To demonstrate detection limits of the assay, dilution studies must be performed on (a) at least three positive samples with high concentrations, and (b) Paul Ehrlich Institute (PEI) Reference Preparations for HBeAG and anti-HBe or another recognized standard. Results should be compared to the reference method. Analytical Sensitivity must be determined for both HBeAg and Anti-HBe.

   Acceptable ranges for analytical sensitvity may vary depending on the type of method used. Normally, serial two-fold dilutions are prepared, and sensitivity may be considered to equal the highest dilution considered reactive.

2) Analytical Specificity - To asses specificity of the assay in the presence of substances that may interfere with the accuracy of the result, the following studies must be performed for both HBeAg and Anti-HBe:

   a. Anticoagulant study: if the manufacturer claims that the device is suitable for either plasma or serum, a

minimum of 10 positive and 10 negative patient samples should be collected as serum and plasma (for all anticoagulants claimed). For example:

Anticoaogulant Study

Sample	#Tested	Mean (Test)	Mean (Reference)
Serum	10	.0710	.0720
Heparin	10	.0708	.0714
EDTA	10	.0720	.0726
Citrate	10	.0718	.0725

(Results may also be reported as mean absorbance, optical density (O.D) or counts per minute (CPM), depending on methodology used. Standard deviation S.D.) and coefficients of variation (C.V.) must be calculated.)

b. Hemolysis, lipemia study: if the manufacturer claims that neither condition affects the assay, a minimum of 20 samples each, including both positive and negative samples, should be assayed.

c. Cross-Reactivity studies: the manufacturer should assay a minimum of 10 patient specimens with documented presence of antibodies to:

Epstein Barr Virus (EBV), Cytomegalovirus (CMV), Human Immunodeficiency Virus (HIV), HBV, HCV, HDV, Rheumatoid factor (RF) and antinuclear antibodies (ANA).

The manufacturer should define how reactivity was determined, and provide levels when availabe. Sera selected should include specimens with high levels of antibody.

For example:

Specificity Study

Sample	#Tested	#Reactive (Test)*	# Reactive (Reference)
EBV	10	0	0
HIV	10	4	5
RF	20	2	2
HEV	10	1	1

* Reactive results are reported as per cent interference, not as percent agreement with the reference method, e.g., 4/10 or 40% of HIV samples tested exhibited false positive results.

d. Serial Samples Collected During the Course of HBV Infection.
If the manufacturer claims that the assay is also indicated to determine seroconversion, serial samples should be collected from a minimum of five patients diagnosed as acute type B hepatitis. Results should exhibit a decline in HBeAg and an increase in Anti-HBe.

3) Stability Studies - To demonstrate the shelf life claimed in the labeling, the device and/or components should be assayed beyond the specified time limit. Real time data must be submitted, and a minimum of three lots should be assayed.

For example, if 45 days shelf life is claimed for a radioactive assay:

Stability Data Summary

Lot #	Labeling date	Zero	15 days	30 days	46 days
1	01/02/89	01/15	01/30	02/15	03/02/89
cpm (total)		2468	2260	1900	1160
cpm (pos. control)		1248	1130	1080	630

(Note: Results should be within the acceptable range up to the last day claimed for product shelf-life, and should comply with Good Manufacturing Practice (GMP) Part 820 of the CFR.)

4) Reproducibility Studies - To assess the precison of the assay when the same lot is tested at different times and places, reproducibility studies should be performed on a minimum of 5 positive, 5 negative and 5 borderline samples, in triplicate, for three days. Intra-assay, inter-assay, and lot-to-lot reproducibility may be determined at the manufacturer's facility; inter-site reproducibility should be performed at an additional two outside sites. Mean, Standard Deviation, and Coefficient of Variation should be included in the statistical analysis.

For example:

Reproducibility Studies (cpm)

Intra-assay

Sample	Mean	Std. Dev.	CV
negative specimen	261	24.12	8.8%
borderline	1656	30.20	5.4%
positive specimen	11377	570.3	5.1%

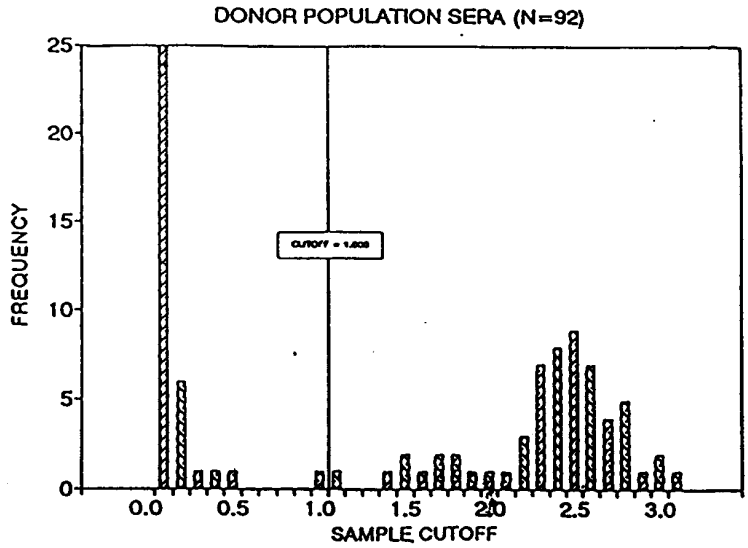
Inter-site

Site	Sample	Kit Lot	Mean	STD	CV
A	High Pos.	1	11684	489	8.9%
	Med. Pos.		7859	198	12.0%
	Borderline		1789	59	14.8%
B	High Pos.	1	11549	479	9.2%
	Med. Pos		7921	189	12.5%
	Borderline		1583	47	13.9%
C	High Pos	1	11765	492	8.9%
	Med. Pos		7529	193	12.4%
	Borderline		1640	87	15.9%

(Note: CV's of 15% are normal for assays of this type; although 20% is acceptable in assays with low counts.)

5) Cutoff Studies - In order to determine accuracy near the cutoff value in qualitative assays, manufacturers are expected to perform cutoff studies. A well-defined clinical population should be used; [i.e., positive population = clinical diagnosis, other hepatitis markers; negative population = clinically healthy and HBsAg (-)]. Methods used to establish cutoff values must be explained. Histograms displaying the distribution of results for positive versus negative samples in the study should also be included.

For example:



B. CLINICAL STUDIES

Studies should be performed at three separate sites in different geographic locations (one site could be the manufacturer's facility; the other two must operate independent of the manufacturer). While foreign data are acceptable, at least one site must be in the U.S. because of differences in the prevalence of HBV in foreign and U.S. populations. Clinical studies should be documented with patient histories which should include the clinical diagnosis and how the diagnosis was confirmed. If the device is indicated for "monitoring of disease", patients must be followed for disease progression in relation to results obtained with this device.

Clinical protocol should clearly address how discrepancies with the reference method are resolved. Explanations of differences should include other serum enzyme levels assayed, other hepatitis markers tested or other assay used to resolve discrepant results. Data must be presented for the initial test results and the repeat test.

Each study should include a statistical analysis and a summary of the results, and conclusions drawn from the study. Studies done in clinical trials should include:

1. Clinical Sensitivity - A minimum of 200 patients positve for HBeAg and 200 patients previously diagnosed as positive for anti-HBe should be assayed at each center. Both the assay for the detection of HBeAg and the assay for Total Anti-HBe should include symptomatic, acute, chronic, and convalescent patients, with explanation of how these populations were defined. Serial samples collected during the course of disease must also be

included; library or retrospective samples may be used. A summary of results may be exhibited as follows or in a similar format:

Specimens	Number Tested	R T - -	R T - +	R T + -	R T + +
Suspected HBV	200	15	0	0	185
anti-HBe pos.	50	0	0	0	50
HBeAg pos	50	0	0	1	49

(99.5% Sensitivity when compared to the reference method. R = Reference Method, T = Test Method)

2) Clinical Specificity - A minimum of 500 healthy volunteers testing negative for Total anti-HBe or HBeAg or HBsAg should be assayed at each site. Patient samples known to be reactive for other viral diseases (see Pre-Clinical section) should be included in this study to assess specificity of the assay when testing patients with other diseases that may produce aberrant results.

For example:

Specimens	Number Tested	R T - -	R T - +	R T + -	R T + +
Normal Donors	500	450	2	2	46
Other diseases	50	48	0	0	2

(99.5% Specificity when compared to the reference method)

4. LABELING CONSIDERATIONS:

The PMA should conform to part 814 of the CFR and all labeling should conform to section 809.10 of the CFR and be appropriate and complete for a device of its kind.

The caution statement for prescription devices should appear on the first page of the package insert.

1) Each device will have the same intended use as the generic device (see definition); however, each manufacturer must specify in the package labeling:

   a. The specific class(es) of antibody detected with the device, e.g., IgG, IgM.

b. The specific technology/methodology upon which the device is based.

c. The types of patient samples to be used with the device.

2) Appropriate warnings and contraindications no less stringent than in previous submissions, concerning use of biological hazards, radioactive labeling, carcinogens, etc. Some examples are:

   a. Biological hazards: for devices containing or made from human blood or serum, the insert must state whether or not the human materials have been tested for the presence of HIV antibody as well as hepatitis B surface antigen. (809.10(b)(5)

   b. Radioactive material such as Iodine-125, Cobalt, Tritium must be labeled with instructions for receipt, possession, disposal and storage according to U.S. Nuclear Regulatory Commission (NRC) licensure guidelines and state and local regulations.

   c. Carcinogens and toxic chemicals such as sodium azide, which causes the formation of potentially explosive lead or copper azide, must be so labeled.

3) The Interpretation of Results section should clearly define a positive or negative result with cutoff levels where appropriate. If there are equivocal zones or results that should be repeated, this must be clearly stated along with guidelines to follow. Tables with representations of the serological patterns observed for HBsAg, HBeAG, anti-HBc, anti-HBe, and anti-HBs during acute and chronic infection should be included.

4) The Performance Characteristics section must include a summary of data from clinical trials upon which the performance of the test is based. Data must be presented for the initial test results and the repeat test results with explanations.

5) Bibliography should include pertinent up-to-date references for information cited in the text and any other references related to the subject matter.

5. BIBLIOGRAPHY

1) Mandell GL, Douglas RG, Bennett JE, editors. Principles and Practice of Infectious Diseases. 3rd ed. New York: Churchill Livingstone Inc; 1990, 1985.

2) Balows A, Hausler WJ Jr, Herrmann KL, Shadomy HJ,

editors. Manual of Clinical Microbiology. 5th ed. Washington, D.C.: 1991.

3) Fody EP, Johnson DF. The serological diagnosis of viral hepatitis. J Med Tech 1987; 4:54-58.

4) Hojvat SA. Diagnostic tests for viral hepatitis. Clin Microbiol News 1989; Vol. II, No. 5.