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HBsAg Performance Improvement of Novel MAS™ Infectious Controls for Serology Diagnostic Tests

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Abstract

Background: Hepatitis B is a liver infection caused by the Hepatitis B virus. While preventable by vaccination, Hepatitis B remains prevalent across the globe. While prevalence is low in many areas of the world, there are still regions in which the prevalence is considered to be high. Hepatitis B surface antigen (HBsAg) can be one of the first indicators of Hepatitis B infection, and it is a commonly screened diagnostic test to determine if a patient is immune, susceptible, or has acute or chronic HBV infection. While HBsAg is a critical marker in diagnosing a patient, it presents unique challenges. The Thermo Scientific™ MAS™ Omni™ Infectious BSI Positive Control Panel* is a multi-constituent IVD control containing anti-HIV-1/2, anti-HBc, anti-HCV, and anti-HTLV I/II antibodies, as well as Hepatitis B surface antigen (HBsAg) in a human plasma-based matrix. Development and Stability Studies were conducted to evaluate and ensure optimal analyte performance throughout the shelf-life duration. As this is a multi-constituent control, the HBsAg challenges must be adequately addressed to ensure the integrity and reliability of the results of all analytes. Two specific areas of focus are HBsAg instability at increased temperature and HBsAg-Fibrinogen interaction in plasma products. We report here the continued development and assessment of HBsAg in the MAS™ Omni Infectious Controls family.

Methods: The BSI Positive Control Panel was evaluated with IVD assays to assess Shelf-Life and demonstrate the effect of elevated temperature and fibrinogen on HBsAg. Shelf life of the products was determined by real-time, accelerated, open vial, and in-use stability monitoring. Additionally, open-vial stability monitoring demonstrates the relationship between HBsAg and fibrinogen within the control products. Between Month 0 and 12, samples were run neat without processing. At Month 12, samples were centrifuged to verify the validity of the open-vial stability claim.

Results: The evaluation of the BSI Positive Control Panel demonstrates the effects of temperature and fibrinogen on HBsAg. The Real-Time and Accelerated stability studies combine to demonstrate the effect of elevated temperatures on HBsAg within the multi-analyte control. The Open-Vial stability studies completed at 0 and 12 months combine to demonstrate the effect of fibrinogen on HBsAg within the multi-analyte control.

Conclusions: The MAS™ Omni Infectious control products will perform optimally provided the proper sample processing and storage conditions are implemented. Improper storage at elevated temperatures will result in degradation at an increased rate for the HBsAg present in the sample. Additionally, it is imperative that the proper raw materials are chosen in the production of the multi-analyte controls. When using plasma products, it is best to use defibrinated plasma. For extra precaution, raw material should be centrifuged and filtered prior to production to avoid degradation due to fibrinogen. Reliable, high-quality controls are vital in ensuring that clinical diagnostic assays are functioning properly.

*Availability of product in each country depends on local regulatory marketing authorization status

Introduction

Designing and developing a reliable and consistent multi-constituent control may present one with an array of complexities and challenges. These challenges may include analyte compatibility, optimal storage temperature, individual analyte stability, etc. The MAS Omni Infectious BSI Positive Control Panel products are multi-constituent controls containing anti-HIV ½, anti-HBc, anti-HCV, and anti-HTLV I/II antibodies, as well as Hepatitis B surface antigen (HBsAg) in a human plasma-based matrix. HBsAg is a critical marker in detecting the presence of the Hepatitis B virus, but it presents unique challenges within this multi-constituent control including:

- Instability at elevated temperatures
- Decreased detection due to anti-HBs and/or fibrinogen

In order to produce a high-quality product, extra pre-cautions may be needed to ensure consistent, reliable, and stable performance.

Materials and methods

The evaluation of HBsAg through Real-Time, Accelerated, and Open-Vial stability has been completed on the Abbott Architect for the MAS™ Omni Infectious BSI Positive Control – For Use with Siemens Assays.

MAS™ Product Description	Analyte	Pack Size	Shelf Life @ 2-8 ºC	Open Vial Stability @ 2-8 ºC
Thermo Scientific™ MAS™ Omni Infectious BSI Positive Control Panel – For Use with Siemens Assays (10027480-U)	anti-HIV 1/2 anti-HTLV I/II HBsAg anti-HBc anti-HCV	5 X 5 mL	12 months	60 days

Table 1. MAS™ Omni Infectious BSI Positive Control Panel – For Use with Siemens Assays

Open-Vial Stability

Open-Vial Stability has been completed to substantiate a 60 day Open-Vial Stability claim. To ensure that the open-vial claim is valid through the duration of the shelf-life, open-vial studies have been tested at 0 Months (Uncentrifuged) and 12 months (Centrifuged). Additionally, the studies demonstrate the negative impact of fibrinogen on HBsAg.

Real-Time and Accelerated Stability

Real-Time and Accelerated Stability (23C and 37C) have been completed to substantiate a 12-month Shelf-Life claim. Additionally, the studies demonstrate the negative impact of elevated temperature on HBsAg.

Acceptance Criteria

To meet the requirements of each study, all samples must remain positive (>1.0 S/CO).

Results

Open-Vial Stability

MAS™ Omni Infectious BSI Positive Control Panel - For Use with Siemens Assays					
HBsAg - Open Vial Stability (2-8° C)					
0 Month			12 Month		
Timepoint (Day)	Mean (S/CO)	% Change	Timepoint (Day)	Mean (S/CO)	% Change
0	6.41	-	0	4.41	-
10	6.14	-4.1	10	4.60	4.3
20	5.81	-9.3	20	4.65	5.4
30	5.58	-12.8	30	4.75	7.6
40	5.49	-14.3	40	4.63	4.9
50	5.41	-15.6	50	4.66	5.5
60	5.27	-17.7	60	4.58	3.8
65	5.66	-11.6	65	4.53	2.6

Table 2. MAS™ Omni Infectious BSI Positive Control Panel – For Use with Siemens Assays HBsAg Open Vial Stability

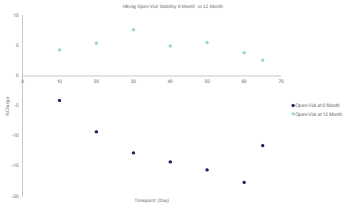

Figure 1. MAS Omni Infectious BSI Positive Control Panel – For Use with Siemens Assays HBsAg Open Vial Stability

Real-Time and Accelerated Stability

MAS™ Omni Infectious BSI Positive Control Panel - For Use with Siemens Assays			
HBsAg Real-Time Stability (2-8° C)			
Equivalent Timepoint (Month)	Timepoint (Month)	Mean (S/CO)	%Change
0	0	7.19	-
3	3	6.09	-15.3
6	6	6.43	-10.6
12	12	5.02	-30.3
13	13	5.35	-25.6
HBsAg 23° C Accelerated Stability			
Equivalent Timepoint (Month)	Timepoint (Day)	Mean (S/CO)	%Change
0	0	7.19	-
3	24	4.97	-30.9
6	49	4.31	-40.1
12	98	3.71	-48.4
13	106	3.58	-50.2
HBsAg 37° C Accelerated Stability			
Equivalent Timepoint (Month)	Timepoint (Day)	Mean (S/CO)	%Change
0	0	7.19	-
3	9	4.44	-38.3
6	19	3.33	-53.7
12	37	2.30	-68.0
13	40	2.18	-69.7

Table 3. MAS™ Omni Infectious BSI Positive Control Panel – For use with Siemens Assays HBsAg Open-Vial Stability

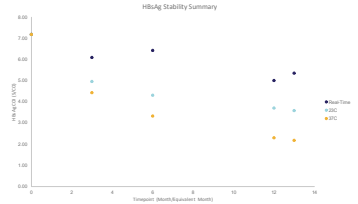

Figure 2. MAS™ Omni Infectious BSI Positive Control Panel – For Use with Siemens Assays HBsAg Real-Time and Accelerated Stability

Conclusions

- In any plasma-based control material, there is the possibility that the sample may be affected by fibrinogen. To prevent a negative impact from fibrinogen, the simple solution would be to ensure all raw materials have been defibrinated. Even with this process, small amounts of fibrinogen may remain. While fibrinogen may not be visually detectable immediately, it could still have an impact on the samples. The HBsAg Open-Vial Stability studies appear to show more adequate and consistent performance at 12 Months as opposed to 0 Months.
  - 0 Month Open-Vial Stability – The 0-month samples were tested unprocessed, and there was no visual presence of fibrinogen in the samples. However, the HBsAg performance was decreased by ~18% at 60 days.
  - 12 Month Open-Vial Stability – The 12-month sample were tested centrifuged for 10 minutes at 4000g prior to Day 0 testing due to visual presence of fibrinogen. In addition to HBsAg performance, the presence of fibrinogen may create sample aspiration errors on clinical analyzers. With this pre-treatment, the HBsAg performance remains stable increasing by ~4% at 60 days.
- HBsAg is unstable at elevated temperatures as shown by the Real-Time and Accelerated stability study data. Over the course of 13 months, the results show a decrease of ~26% at 2-8C, ~50% at 23C, ~70% at 37C. The results show that the rate of degradation is greatly increased with the greater elevation in temperature.
- The MAS™ Omni Infectious BSI Positive Control Panel – For Use with Siemens Assays meets the requirements of all stability studies with no impact on product risk, safety, or efficacy when used and stored properly.

References

1. Mayer, T.K., Vargas, R.L., Knebel, A.E. et al. Hepatitis B assays in serum, plasma, and whole blood on filter paper. *BM Clin Pathol* **12**, 8 (2012). https://doi.org/10.1186/1472-6890-12-8.
2. Vanstapel, M.J., De Wolf-Peeters, C., De Vos, R. and Desmet, V.J. (1984), Hepatitis B surface antigen (HBsAg) – fibrinogen interaction. Liver, 4: 148-155. https://doi.org/10.1111/j.1600-0676.1984.tb00920.x

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Trademarks

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