施马伦贝格病毒荧光 RT-PCR 检测试剂盒

背景和原理 Backgrounds and Princinples

施马伦贝格病毒（schmallenberg virus，SBV）是一种由库蠓传播的虫媒病毒，是一种新型的布尼亚病毒。SBV 可感染牛、绵羊和山羊等反刍动物，病畜感染后表现出发热、腹泻及乏力等症状，怀孕母畜感染后还可见流产、胎儿畸形等症状，严重危害疫情地区畜牧业安全。SBV 与其它布尼亚病毒目成员结构相似，存在节段重组和基因漂移的特点，这使得 SBV 检测较为困难。我国尚无该病疫情报道。

本试剂盒以 SBV-S 为靶基因，采用 Taqman 水解探针工作原理，用于检测样品中的施马伦贝格病毒核酸，试剂盒内包含方法质控品、裂解液、反应液、酶混合物、阳性对照、阴性对照等所有实时荧光 RT-PCR 所需的反应成分，用户只需提取核酸加入模板即可上机进行检测。

特点 Characteristics

- 适用于施马伦贝格病毒检测；
- 敏感性(Dse)：96.43%；特异性(Dsp)：93.94%；
- 操作简便、快速。

作用用途 Function Purpose

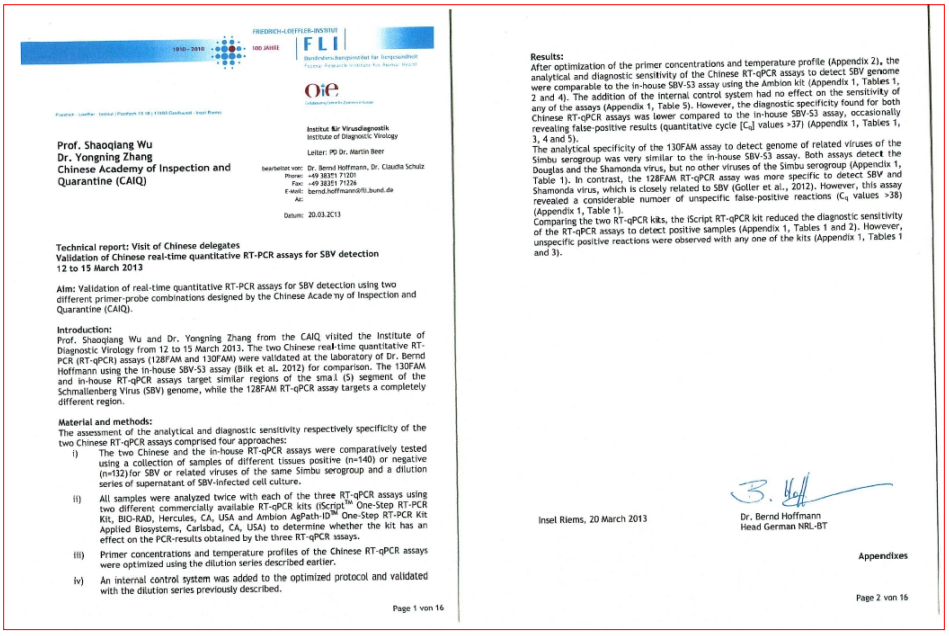
- 用于检测牛羊等组织、血液、精液等中 SBV。适用于施马伦贝格病相关检测、监测与流行病学调查。

应用案例 The Applications case

案例

该检测试剂盒经德国 FLI 所国家兽医实验室进行评价，具有良好的敏感性（可达 1TCID$_{50}$）、特异性，适用于施马伦贝格病毒相关核酸检测。

该检测试剂盒配套施马伦贝格病毒标准物质，组织施马伦贝格病能力验证，满足施马伦贝格病检验检测技术需求。



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Technical report: Visit of Chinese delegates
Validation of Chinese real-time quantitative RT-PCR assays for SBV detection
12 to 15 March 2013

Aim: Validation of real-time quantitative RT-PCR assays for SBV detection using two different primer-probe combinations designed by the Chinese Academy of Inspection and Quarantine (CAIQ).

Introduction:
Prof. Shaoqiang Wu and Dr. Yongning Zhang from the CAIQ visited the Institute of Diagnostic Virology from 12 to 15 March 2013. The two Chinese real-time quantitative RT-PCR (RT-qPCR) assays (128FAM and 130FAM) were validated at the laboratory of Dr. Bernd Hoffmann using the in-house SBV-S3 assay (Bilk et al. 2012) for comparison. The 130FAM and in-house RT-qPCR assays target similar regions of the small (S) segment of the Schmallenberg Virus (SBV) genome, while the 128FAM RT-qPCR assay targets a completely different region.

Material and methods:
The assessment of the analytical and diagnostic sensitivity respectively specificity of the two Chinese RT-qPCR assays comprised four approaches:
i) The two Chinese and the in-house RT-qPCR assays were comparatively tested using a collection of samples of different tissues positive (n=140) or negative (n=132) for SBV or related viruses of the same Simbu serogroup and a dilution series of supernatant of SBV-infected cell culture.
ii) All samples were analyzed twice with each of the three RT-qPCR assays using two different commercially available RT-qPCR kits (iScript™ One-Step RT-PCR Kit, BIO-RAD, Hercules, CA, USA and Ambion AgPath-ID™ One-Step RT-PCR Kit Applied Biosystems, Carlsbad, CA, USA) to determine whether the kit has an effect on the PCR-results obtained by the three RT-qPCR assays.
iii) Primer concentrations and temperature profiles of the Chinese RT-qPCR assays were optimized using the dilution series described earlier.
iv) An internal control system was added to the optimized protocol and validated with the dilution series previously described.

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Results:
After optimization of the primer concentrations and temperature profile (Appendix 2), the analytical and diagnostic sensitivity of the Chinese RT-qPCR assays to detect SBV genome were comparable to the in-house SBV-S3 assay using the Ambion kit (Appendix 1, Tables 1, 2 and 4). The addition of the internal control system had no effect on the sensitivity of any of the assays (Appendix 1, Table 5). However, the diagnostic specificity found for both Chinese RT-qPCR assays was lower compared to the in-house SBV-S3 assay, occasionally revealing false-positive results (quantitative cycle [C_q] values >37) (Appendix 1, Tables 1, 3, 4 and 5).
The analytical specificity of the 130FAM assay to detect genome of related viruses of the Simbu serogroup was very similar to the in-house SBV-S3 assay. Both assays detect the Douglas and the Shamonda virus, but no other viruses of the Simbu serogroup (Appendix 1, Table 1). In contrast, the 128FAM RT-qPCR assay was more specific to detect SBV and Shamonda virus, which is closely related to SBV (Goller et al., 2012). However, this assay revealed a considerable numoer of unspecific false-positive reactions (C_q values >38) (Appendix 1, Table 1).
Comparing the two RT-qPCR kits, the iScript RT-qPCR kit reduced the diagnostic sensitivity of the RT-qPCR assays to detect positive samples (Appendix 1, Tables 1 and 2). However, unspecific positive reactions were observed with any one of the kits (Appendix 1, Tables 1 and 3).

Insel Riems, 20 March 2013

Dr. Bernd Hoffmann
Head German NRL-BT

Appendixes

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产品名称	货号	产品规格/盒
施马伦贝格病毒荧光 RT-PCR 检测试剂盒	AQ-RM-09	48 头份/盒