

Highest Quality and Reproducibility in CMV & HSV Testing

¹sYnlab Labordienstleistungen, Zur Kesselschmiede 4, 92637 Weiden, Germany

²chemagen AG, Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany

Abstract: Diagnostics of viral pathogens such as Cytomegalovirus (CMV) or the different types of Herpes Simplex Viruses (HSV) have to meet demands of the European Quality Standards. Each year laboratories in Europe are invited to take part in the QCMD, the Quality Control for Molecular Diagnostics. This study is organized in collaboration with the European Society for Clinical Virology and the European Society for Clinical Microbiology & Infectious Diseases.

A crucial step in pathogen diagnostics is the efficient isolation of viral nucleic acids. Comparing given values with own results demonstrates if your entire laboratory workflow processes are standardized.

In 2008 the sYnlab laboratory in Weiden, Germany, participated in this standardized QC to test their automated sample preparation system in combination with their in-house Real Time PCR methods. Plasma samples from CMV as well as from HSV/VZV positive and negative patients were analyzed. The results of sYnlab are 100 % comparable to the given QC values.

Materials & Methods: The lyophilized QC samples had to be diluted in a declared amount of H₂O first. Then the chemagic Magnetic Separation Module I (chemagic MSM I) was used for automated DNA isolation from 300 µl plasma/H₂O using the chemagic Viral DNA/RNA Kit special. The subsequent Real Time PCR (Figure 1) was performed with 1/10 of the eluates using an in-house PCR system.

Results: The comparison of the results given in the QC and the ones performed by sYnlab, Weiden, correlated to 100 % (Table 1).

QCMD					sYnlab, Weiden	
Panel Code	Matrix	Sample Contents	Sample Conc.	Expected Result	CP	Result
CMV08-01	VTM*	CMV (Strain AD 169)	216272	Positive	26.80	Positive
CMV08-02	Plasma	CMV (Strain AD 169)	962	Positive	34.77	Positive
CMV08-03	Plasma	CMV Negative		Negative		Negative
CMV08-04	Plasma	CMV (Strain AD 169)	4055	Positive	32.66	Positive
CMV08-05	Plasma	CMV (Strain AD 169)	236	Positive	34.80	Positive
CMV08-06	Plasma	Negative		Negative		Negative
CMV08-07	Plasma	CMV (Strain AD 169)	21038	Positive	30.20	Positive
CMV08-08	VTM*	CMV (Strain AD 169)	2182730	Positive	23.12	Positive
CMV08-09	Plasma	CMV (Strain AD 169)	869	Positive	34.46	Positive
CMV08-10	VTM*	CMV (Strain AD 169)	22542	Positive	30.32	Positive

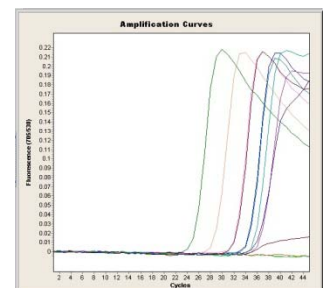


Figure 1:
RT-PCR. Application curves of 10 QC CMV samples, an internal positive and an internal negative control.

Table 1:
Comparison of sYnlab's results to given QC values.
*VTM: Virus Transport Medium = Dulbecco's Modified Eagle's Medium and Foetal Calf Serums



Herpes Simplex Virus Testing

The viral Nucleic Acids of Herpes Simplex Virus (HSV) Type 1 and 2 and Varizella Zoster Virus (VZV) diluted in DMM/BSA were isolated with the chemagic MSM I. The subsequent Real Time PCR (Figure 2) confirmed the QCMD values.

QCMD					sYnlab, Weiden	
Panel Code	Matrix	Sample Contents	Stock Dilution	Sample Status	CP	Result
HSV08-01	DMM/BSA	HSV Type 1	1.1x10 ⁻⁴	Strong Positive	30.67	Strong Positive
HSV08-02	DMM/BSA	HSV Type 2	1.1x10 ^{-6.5}	Positive	>41.00	Positive
HSV08-03	DMM/BSA	HSV Type 1	1.1x10 ⁻²	Strong Positive	23.32	Strong Positive
HSV08-04	DMM/BSA	HSV Type 2	1.1x10 ⁻⁵	Strong Positive	34.30	Strong Positive
HSV08-05	DMM/BSA	HSV Neg. Medium		Negative		Negative
HSV08-06	DMM/BSA	VZV (Ellen)		Negative		Negative
HSV08-07	DMM/BSA	HSV Type 1	1.1x10 ⁻³	Strong Positive	26.80	Strong Positive
HSV08-08	DMM/BSA	HSV Type 2	1.1x10 ^{-6.5}	Positive	36.80	Positive
HSV08-09	DMM/BSA	HSV Type 1	1.1x10 ^{-5.5}	Positive	38.29	Positive
HSV08-10	DMM/BSA	HSV Type 2	1.1x10 ^{-5.5}	Positive	36.64	Positive
HSV08-11	DMM/BSA	HSV Neg. Medium		Negative		Negative
HSV08-12	DMM/BSA	HSV Type 2	1.1x10 ⁻²	Strong Positive	23.73	Strong Positive

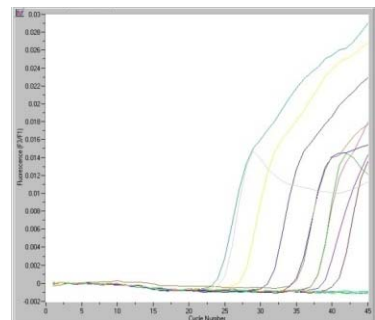


Figure 2
RT PCR. Application curves of 12 QC HSV samples, an internal positive and an internal negative control.

Table 2:
Comparison of sYnlab's results to given QC values..

Conclusion: The isolation of CMV or HSV DNA with the chemagic MSM I and the chemagic Viral DNA/RNA special Kit provide viral DNA of highest quality as well as in sufficient quantity. Due to outstanding reproducibility, sYnlab's in-house Real Time PCR in combination with chemagen's automated sample preparation meet the standardized quality testings criteria.