

# Titel: ZAS PRÄS Ringtrial SRS, EV 1.5

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## Collaborative study of isolation and RT-PCR of enteric viruses using a synthetic standard

### Abstract

A collaborative study was performed to compare the sensitivities of isolation and RT-PCR analysis of various enteric viruses in different laboratories. As Norwalk like viruses (NLV or SRS) are not cultivable and no standards are available a synthetic standard was Type I+II+III created containing all sites of the applied RT-PCR-systems. Using this standard it was possible to compare the sensitivities of the PCR systems and to estimate the concentration of the standard compared to a well-characterized poliovirus (Sabin). The sensitivities of Enterovirus and SRS II RT-PCR were 2 TCID<sub>50</sub> (Poliovirus equivalent). It was shown that SRS I RT-PCR was 1000 less sensitive.

### Introduction

This collaborative study used an artificial and calibrated RNA-standard together with a Poliovirus strain (Vaccine) as reference material. The performance of filtration, RNA-isolation and RT-PCR of Enterovirus and SRS Type I and Type II was evaluated. Because the iSt EnV contains the sites of all applied RT-PCR systems, the calibration is valid for all applied RT-PCR systems.

### Material and Methods

#### Reference material, Preparation, Packaging, Labeling, Distribution and Stability

The reference material was prepared as a stock solution, which was homogenized carefully and aliquoted in tubes (RNase and DNase free). The material was shipped on dry ice and the participants had to confirm the correct status of the material after arrival. The first tube contained the iSt EnV and the second tube contained a poliovirus solution (Sabin Type I+II+III, Smithkline Beecham). The water for the filtration was chosen by the participants.

The internal Standard EnV (iSt EnV, RNA) is commercially available from Biosmart GmbH (patent pending, www.biosmart.ch). The calibration by real-time RT-PCR (LightCycler) gave the following equivalence: 10 µl E-10 (Lot 150707) equal to 1.6 TCID<sub>50</sub> (Poliovirus Sabin).

#### Filtration and RT-PCR

The methodology is based on the Publication of **M. Gilgen et al. 1997**. Participants were instructed precisely how to dilute the reference material in 1 liter of water. They were free to choose the filtration and RNA isolation method.

Isolation No	Preparation	Poliovirus	iSt EnV
0	Filtration of 1 liter of water	no	no
1	Filtration of 1 liter of water	284 TCID <sub>50</sub>	10 µl iSt EnV E-10
2	Filtration of 1 liter of water	28,4 TCID <sub>50</sub>	10 µl iSt EnV E-10
3	Filtration of 1 liter of water	2,84 TCID <sub>50</sub>	10 µl iSt EnV E-10
4	Filtration of 1 liter of water	0,284 TCID <sub>50</sub>	10 µl iSt EnV E-10
5	Filtration of 1 liter of water	0,0284 TCID <sub>50</sub>	10 µl iSt EnV E-10
6	Filtration of 1 liter of water	0,00284 TCID <sub>50</sub>	10 µl iSt EnV E-10
7	only RNA Isolation method (Qiagen)	no	10 µl iSt EnV E-6, 16000 TCID <sub>50</sub>
8	1:10 dilution of eluate of No 7	no	10 µl iSt EnV E-6, 1600 TCID <sub>50</sub>
9	1:100 dilution of eluate of No 7	no	10 µl iSt EnV E-6, 160 TCID <sub>50</sub>
10	1:1000 dilution of eluate of No 7	no	10 µl iSt EnV E-6, 16 TCID <sub>50</sub>
11	1:10'000 dilution of eluate of No 7	no	10 µl iSt EnV E-6, 1,6 TCID <sub>50</sub>
12	1:100'000 dilution of eluate of No 7	no	10 µl iSt EnV E-6, 0,16 TCID <sub>50</sub>
13	1:1 Mio dilution of eluate of No 7	no	10 µl iSt EnV E-6, 0,016 TCID <sub>50</sub>

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## Results and Discussion

Four of six laboratories were able to perform the collaborative study and send results. Two of the laboratories produced two sets of results.

**Table 2** shows the results of the **Enterovirus filtration and RT-PCR**. None of the isolation negative controls showed a false positive signal (Isolation No 0). Laboratory A and C showed consistent results of the filtration and RT-PCR. Laboratory B and D showed less consistent results in this experiment. The detection limit is estimated to be at 2.8 TCID<sub>50</sub> (Isolation No 3). All laboratories were able to discriminate between the iSt EnV and poliovirus signal (competitive PCR). The filtration based on real-time PCR (LightCycler) was confirmed within one decade (equivalence point around No 4).

### Table 2: Enterovirus RT-PCR

Legend: de: detected / total, nd: not detected

	Isolation No 0	Isolation No 1	Isolation No 2	Isolation No 3	Isolation No 4	Isolation No 5	Isolation No 6	Isolation No 7
<b>A</b> Enterovirus signal	0/2	2/2	2/2	2/2	2/2	2/2	1/2	0/2
iSt EnV Signal	0/2	0/2	0/2	0/2	0/2	1/2	1/2	2/2
<b>B</b> Enterovirus signal	0/1	1/1	0/1	1/1	0/1	0/1	1/1	0/1
iSt EnV Signal	0/1	0/1	1/1	1/1	1/1	1/1	0/1	1/1
<b>C</b> Enterovirus signal	0/2	2/2	2/2	2/2	2/2	1/2	0/2	0/2
iSt EnV Signal	0/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
<b>D</b> Enterovirus signal	0/1	1/1	1/1	1/1	0/1	0/1	1/1	nd
iSt EnV Signal	0/1	1/1	1/1	1/1	1/1	1/1	1/1	nd
<b>Sum A-D</b> Enterovirus signal	0/6	6/6	5/6	6/6	4/6	3/6	3/6	0/5
EnV signal	0/6	3/6	4/6	4/6	4/6	5/6	4/6	5/5

The **SRSI and SRSII RT-PCR (Table 3 and 4)** showed for all laboratories (except Lab B that did not perform the RT-PCR for SRSI) no false positive of the 0-control (Isolation No 0). After receiving the results the reference material was analyzed again to show the stability of the material over the time. This could be confirmed. The following statements assume that the RNA-isolation procedure for all viruses is equally efficient. The sensitivity for SRSI was estimated to be at 1600 TCID<sub>50</sub>. The sensitivity for SRSII was at 1.6 TCID<sub>50</sub>.

For Poliovirus 100-1000 TCID<sub>50</sub> is equal to 1 Human infective dose (HID), **Fields 1996**. For Norwalk and other viruses it is speculated to be lower. Future data have to be generated to answer this question. For analytical purposes, the sensitivities for Enteroviruses and SRSII may be satisfying. The iSt EnV has proven its usefulness to compare RT-PCR methods where no certified cultivatable standards are available.

### Table 3: SRSI RT-PCR

Legend: de: detected / total, nd: not detected

Isol No 0	Isol No 1	Isol No 2	Isol No 3	Isol No 4	Isol No 5	Isol No 6	Isol No 7	Isol No 8	Isol No 9	Isol No 10	Isol No 11	Isol No 12	Isol No 13
Lab A	0/2	0/2	0/2	0/2	0/2	0/2	2/2	2/2	2/2	1/2	0/2	0/1	0/1

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Lab B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lab C	0/2	0/2	0/2	0/2	0/2	0/2	2/2	2/2	1/2	0/2	0/2	0/2	0/2
Lab D	0/1	0/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1	0/1	0/1	0/1
Summary	0/5	0/5	0/5	0/5	0/5	0/5	5/5	5/5	4/5	1/5	0/5	0/4	0/4

## Table 3: SRSII RT-PCR

de: detected / total, nd: not detected

Isol No 0	Isol No 1	Isol No 2	Isol No 3	Isol No 4	Isol No 5	Isol No 6	Isol No 7	Isol No 8	Isol No 9	Isol No 10	Isol No 11	Isol No 12	Isol No 13
Lab A	2/2	2/2	1/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/1	0/1
Lab B	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
Lab C	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	1/2
Lab D	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	0/1
Summary	6/6	6/6	5/6	6/6	6/6	5/6	6/6	6/6	6/6	6/6	6/6	3/5	1/5

## References

Gilgen et al. 1997, International Journal of Food Microbiology 37, 1997, 189-199  
 Hsueh et al. 1996, Virology third edition, 655-712

## Acknowledgements

Thanks to all the laboratories having participated to this collaborative study.