Use of Recombinant Sindbis Virus Technology (AccuPlex™) to Produce Non-Infectious, Whole Process Controls for Chikungunya, Dengue, Norovirus, MERS, and Zika

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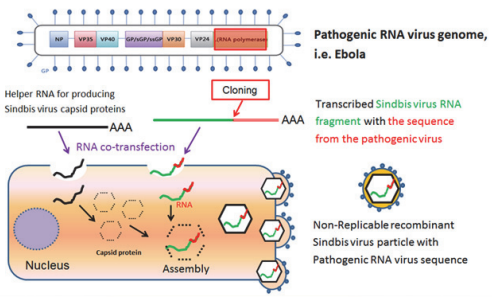
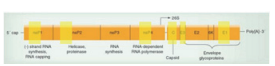
INTRODUCTION

Outbreaks of viral communicable disease and appearance of new viral strains can represent public health emergencies. As diagnostic laboratories and test developers design, manufacture, and validate diagnostic assays to prepare for these threats, positive reference materials are needed. SeraCare has developed five new positive reference materials for chikungunya, dengue-2, norovirus, MERS-CoV, and Zika using recombinant Sindbis virus technology (AccuPlex). The AccuPlex recombinant virus product is a mammalian RNA virus that closely mimics the pathogenic virus in both size and complexity. AccuPlex recombinant viruses are stable 4 °C, room temperature, or elevated temperatures (37 °C).

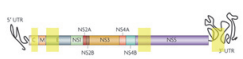
MATERIALS AND METHODS



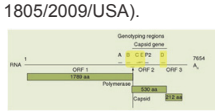
- Recombinant chikungunya reference material contains portions of NSP1, NSP2, NSP4, capsid, E3, and E1 genes, and is based on the sequence of strain IND-06-Guj, originally isolated in India in 2006.



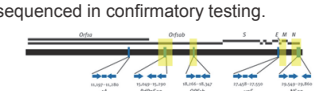
- Recombinant dengue reference material contains portions of 3' UTR, NSP5, capsid, and E1 genes and is based on the sequence of serotype 2.



- Recombinant norovirus reference material contains the conserved region around the ORF1/ORF2 junction including region BCE and region D and is based on sequences from strain GII.4 (Norovirus Hu/GII.4/New Orleans 1805/2009/USA).



- MERS-CoV reference material contains the region upstream of the E protein gene (upE), ORF 1b, and ORF1a; it also contains portions of the RNA-dependent RNA polymerase (RdRp) and (N) genes frequently sequenced in confirmatory testing.



RESULTS AND DISCUSSION

AccuPlex Chikungunya

Digital PCR Quantitation:

Contains:	nmoles	Sequence
Probe	2.5	5'-/56-FAM/ACAGTGGTT/ZEN/TCGTGTGAGGGCTAC/3IABkFQ/-3'
Primer 1	5.0	5'-TGCCATCGGTGTTCCATTTA-3'
Primer 2	5.0	5'-GCCTGGGCTCATCGTTATT-3'

Product Sample	Concentration (cp/mL)	Average (cp/mL)	Stdev	%CV
AccuPlex Chikungunya	8.40E+05	7.94E+05	4.74E+04	5.64%
AccuPlex Chikungunya	7.45E+05			
AccuPlex Chikungunya	7.97E+05			
No Target Control	0			

Functional Testing: altona DIAGNOSTICS RealStar® Chikungunya RT-PCR Kit 1.0 03/2014

Product Sample	Virus CT	Internal control (CT)	FAM Channel Result	Joe Channel Result (IC)
AccuPlex Chikungunya	30.96	32.32	Positive	Positive
AccuPlex Chikungunya	31.3	32.78	Positive	Positive
AccuPlex Chikungunya	31.5	33.82	Positive	Positive
No Target Control	Undet	32.58	Negative	Positive
Kit Positive Control	28.86	Undet	Positive	Negative

AccuPlex MERS

Digital PCR Quantitation:

Contains:	nmoles	Sequence
Probe	2.5	5'-/56-FAM/AAATGCTGG/ZEN/GTATTGGCGGAGACA/3IABkFQ/-3'
Primer 1	5.0	5'-GGCAGGGTGTACCTCTTAATG-3'
Primer 2	5.0	5'-CTGGGAGCCAGTTGCTTAAT-3'

Product Sample	Concentration (cp/mL)	Average (cp/mL)	Stdev	%CV
AccuPlex MERS	2.32E+06	2.74E+06	3.59E+05	13.11%
AccuPlex MERS	2.90E+06			
AccuPlex MERS	2.98E+06			
No Target Control	0			

Functional Testing: altona DIAGNOSTICS RealStar® MERS-CoV RT-PCR Kit 1.0 08/2015

Product Sample	Target	Virus CT	FAM channel
AccuPlex MERS	upE gene	27.58	Positive
AccuPlex MERS	upE gene	28	Positive
AccuPlex MERS	upE gene	28.11	Positive
No Target Control	upE gene	Undet	Negative
Kit Positive Control	upE gene	24.65	Positive
AccuPlex MERS	orf1a gene	29.04	Positive
AccuPlex MERS	orf1a gene	30.06	Positive
AccuPlex MERS	orf1a gene	30.22	Positive
No Target Control	orf1a gene	Undet	Negative
Kit Positive Control	orf1a gene	24.33	Positive

World Health Organization (WHO) recommends two independent PCR assays for confirmation of MERS-CoV cases. Therefore the RealStar MERS-CoV RT-PCR Kit targets a region upstream of the E gene (upE) and an open reading frame 1a (orf1a). AccuPlex MERS Reference Material is positive for both targets.

AccuPlex Dengue

Digital PCR Quantitation:

Contains:	nmoles	Sequence
Probe	2.5	5'-/56-FAM/AACATCTTG/ZEN/AACAGGAGACGCAGGAC/3IABkFQ/-3'
Primer 1	5.0	5'-GAGGGTTCAGGAAAGAGATTGG-3'
Primer 2	5.0	5'-CCGTTGCGTGTGGTTAAATG-3'

Product Sample	Concentration (cp/mL)	Average (cp/mL)	Stdev	%CV
AccuPlex Dengue	2.36E+06	2.35E+06	1.58E+04	0.67%
AccuPlex Dengue	2.36E+06			
AccuPlex Dengue	2.33E+06			
No Target Control	0			

AccuPlex Norovirus GII

Digital PCR Quantitation:

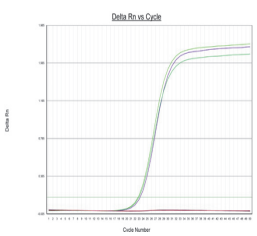
Contains:	nmoles	Sequence
Probe	2.5	5'-/56-FAM/TTTCCTGCG/ZEN/GAGAACTGTGACCC/3IABkFQ/-3'
Primer 1	5.0	5'-TGAAGACCTGAATGGCCTAAC-3'
Primer 2	5.0	5'-GTCCAGTACATTTGCCTGAGTA-3'

Product Sample	Concentration (cp/mL)	Average (cp/mL)	Stdev	%CV
AccuPlex Norovirus GII	2.27E+06	2.30E+06	1.94E+05	8.55%
AccuPlex Norovirus GII	2.51E+06			
AccuPlex Norovirus GII	2.13E+06			
No Target Control	0			

Functional Testing: Primerdesign™ Ltd Norovirus Genogroups 1 and 2 genesig®

Product Sample	Target	Virus CT	Internal control (CT)	Result
AccuPlex Norovirus GII*	RNA dependent RNA polymerase gene	29.86	25.55	Positive
AccuPlex Norovirus GII*	RNA dependent RNA polymerase gene	30.12	25.26	Positive
AccuPlex Norovirus GII*	RNA dependent RNA polymerase gene	30.12	25.56	Positive
No Target Control	RNA dependent RNA polymerase gene	Undet	25.69	

*Tested at a dilution. Primerdesign genesig Advanced Kit assays capsid gene for noro GI and RNA-dependent RNA polymerase gene for GII. Results for GII testing are shown. GI capsid gene was not detected.

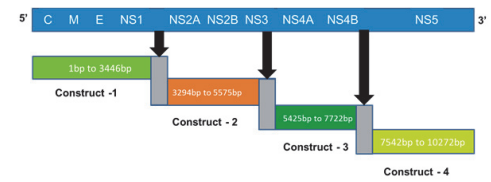


Testing of AccuPlex Norovirus GII by Primerdesign assay. Green/purple amplification curves are for the RNA-dependent RNA polymerase gene assayed for GII. No detection is seen for the capsid gene target assayed for GI (pink lines).

RECENT ADVANCES: AccuPlex ZIKA

Zika virus is transmitted by the bite of an infected mosquito and reportedly causes microcephaly in babies born to infected mothers. Hundreds of unexpected cases of microcephaly have been diagnosed in Brazil and public health officials are trying to determine if Zika infection in utero is the underlying cause. SeraCare developed AccuPlex Zika as a reference material for the development of diagnostic assays and for training and proficiency of public health labs.

Because few assays are currently available for Zika detection, the diagnostic sequence targets were unknown and we felt it was important to have all Zika genomic sequences in the reference material. The Zika genome was divided between four recombinant viruses. In addition to providing added flexibility, the design enhances safety because each recombinant virus only contains a small portion of the Zika genome.



Zika digital PCR assays were designed to the Zika insert sequences in each recombinant virus. Digital PCR analysis was used to control mixing of the recombinant viruses and ensure that each recombinant virus was present in the final preparation at the desired concentration.

Sample	Target for primers/probe	Average (Copies/mL)
AccuPlex Zika Reference Material	Env	1.72E+05
	NS2/NS3	1.22E+05
	NS4	4.07E+05
	NS5	6.88E+05

CONCLUSIONS

- SeraCare has developed AccuPlex recombinant virus reference materials for dengue, chikungunya, MERS, norovirus, and Zika. These reference materials are replication defective and thus are not infectious and ensure added safety for laboratory staff.
- AccuPlex viruses accommodate large inserts (up to ~4,000 bp). This feature allows development of highly multiplexed reference materials that are compatible with tests assaying multiple gene targets. AccuPlex Zika Reference Material shows multiple recombinant viruses can be combined into a single preparation to encompass all genomic sequences of a target pathogen.
- AccuPlex recombinant viruses are highly stable when diluted in plasma or other biological matrices. Ongoing stability studies support at least 20 month stability of virus in plasma at ambient temperature.
- AccuPlex technology can be used to manufacture in-kit-controls or to make 3rd party reference materials for use in clinical labs monitoring assay performance.