A highly specific and sensitive Applied Biosystems™ TaqMan SCID/SMA multiplex assay optimized for dried blood spots

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BACKGROUND

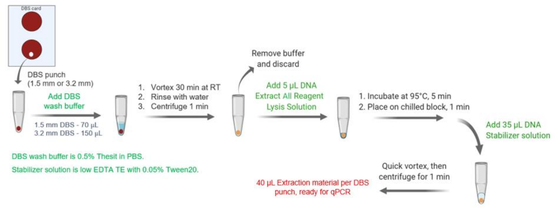
Intensive research in early detection of Severe Combined Immunodeficiencies (SCID) and Spinal Muscular Atrophy (SMA) is essential to discover prevention methods for infants' permanent disabilities or death. In addition to SCID, SMA has been recently added to the US-RUSP list. SMA is a motor neuron disorder caused by mutation in the SMN1 gene, whereas SCID constitutes a series of immune system functionality diseases exhibiting low levels of TREC and/or KREC. We have developed two versions of the TaqMan SCID/SMA multiplex assay (with and without KREC), that permit concomitant detection of both SMA and SCID targets, and each assay includes RNase P gene as an internal genomic control. Additionally, a simple and robust DBS sample preparation method was developed using DNA Extract All Lysis solution (Figure 1).

Multiplex TaqMan™ Assay Fluorescent Label Assignment:

TaqMan™ SCID/SMA assay (A47929): SMN1- FAM, TREC- JUN™, RNaseP (genomic control)- VIC™. Primers and TaqMan® dual labeled probes were synthesized by Thermo Fisher and formulated into a 20X TaqMan™ Assay.

TaqMan™ SCID/SMA Plus assay (A48569): SMN1- FAM, KREC- ABY™, TREC- JUN™, RNaseP (genomic control)- VIC™. Primers and TaqMan™ dual labeled probes were synthesized by Thermo Fisher and formulated into a 20X TaqMan™ Assay.

Figure 1

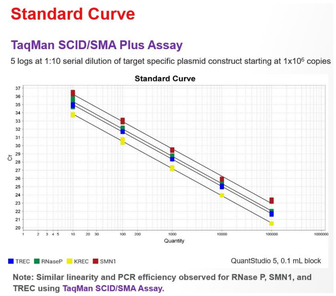


- **Sample preparation workflow duration is ~40 min total with ~35 min hands-off time. When using freshly prepared, routine DBS samples as opposed to desiccated DBS specimens, sample preparation duration is ~20 minutes total.**
- **DBS punch-to-answer duration for 96-well plate format is < 2 hr.**

RESULTS

1. **High linearity and PCR efficiency was observed for all targets (Figure 2). We demonstrate TREC and KREC copy number detection capability of as low as 10 copies per reaction and a LoQ of 25 copies per reaction.**

Figure 2



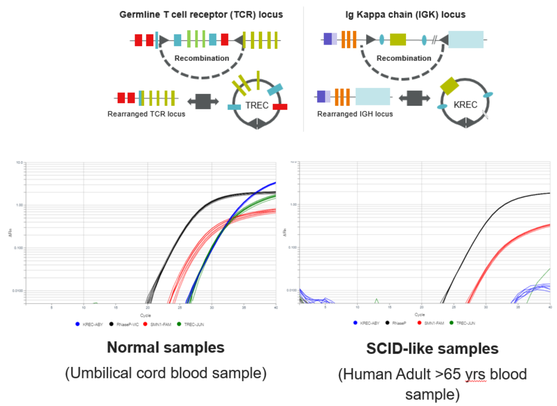
Target	Slope	Y-intercept	R2	Eff (%)
TREC	-3.324	38.319	1	99.9
KREC	-3.319	37.164	0.999	100.1
SMN1	-3.325	39.575	0.997	99.8
RNaseP	-3.278	38.488	0.998	101.8

LoD: 10 copies/reaction for RNase P, SMN1, TREC, KREC

LoQ: 25 copies/reaction for both TREC and KREC

2. **The TaqMan™ SCID/SMA Plus assay is designed to detect the presence of TREC and KREC (Figure 3), while the TaqMan™ SCID/SMA assay is designed to detect the presence of TREC only.**

Figure 3

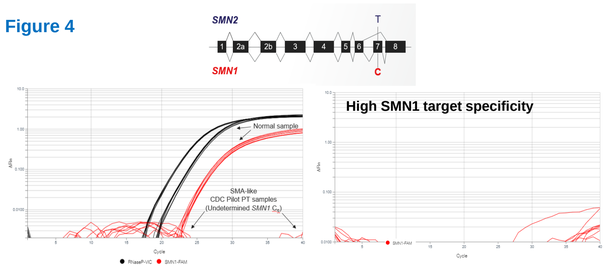


Normal samples
(Umbilical cord blood sample)

SCID-like samples
(Human Adult >65 yrs blood sample)

3. **Both assays are designed to target exon 7 of SMN1 gene and effectively eliminated non-specific detection of the highly similar SMN2 gene (Figure 4 – generated with TaqMan™ SCID/SMA Plus assay).**

Figure 4

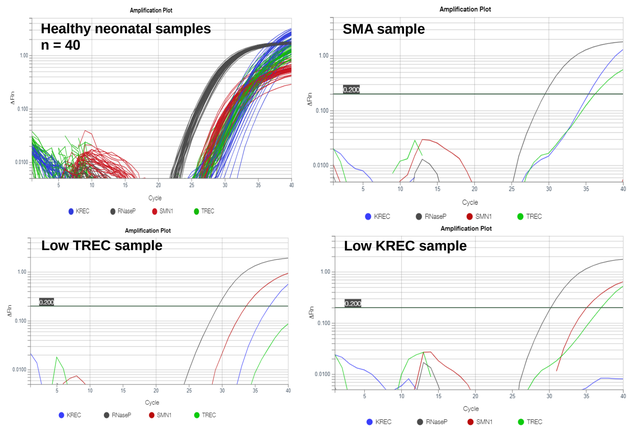


Normal (umbilical cord blood) and **SMA-like samples** (CDC PT samples)

No SMN1 amplification observed when assay was challenged with **3 million copy number of SMN2 gene targets**.

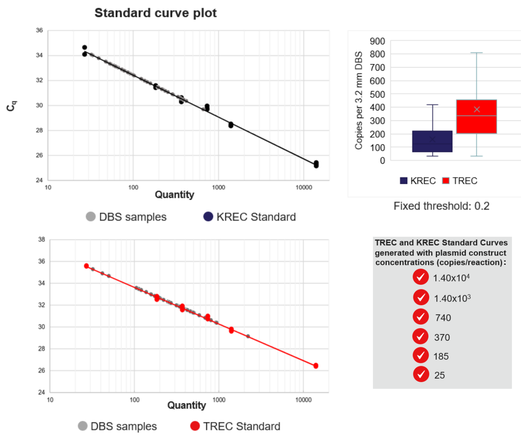
4. **We demonstrate the performance of TaqMan™ SCID/SMA Plus assay with healthy neonatal DBS samples tested at Kumamoto University[1]. Additionally, the assay's high analytical accuracy was evaluated using confirmed SMA positive samples and SCID positive samples containing low levels of TREC/KREC (Figure 5).**

Figure 5



5. **TREC and KREC quantitation: 46 clinical research DBS samples were quantified using the TaqMan™ SCID/SMA Plus Assay. TREC and KREC plasmid constructs were used to generate standard curves. See Figure 6.**

Figure 6



CONCLUSION

In conclusion, we have developed a highly specific, sensitive, and robust multiplex assay for SMA and SCID testing with a rapid and streamlined turnaround workflow to aid further research efforts.

For newborn screening research

Recommended Applied Biosystems™ QuantStudio™ real-time PCR systems (96 and 384 blocks):

- QuantStudio™ 5 system
- QuantStudio™ 6 Flex system
- QuantStudio™ 7 Flex system
- QuantStudio™ Dx system (in RUO mode)
- QuantStudio™ 12K Flex system

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Product links

TaqMan™ SCID/SMA assay:
https://www.thermofisher.com/order/catalog/product/A47927#/A47927

TaqMan™ SCID/SMA Plus assay:
https://www.thermofisher.com/order/catalog/product/A48566#/A48566