



Nested polymerase chain reaction assay for detection of Porcine Astrovirus in pig population

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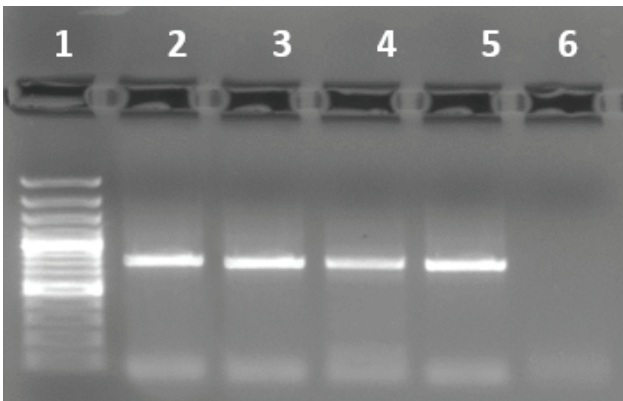


Fig. 1 Gradient PCR using Outer pair of primers
 Lane 1: 100 bp Ladder; Lane 2-5: Gradient temperatures (52°C, 54 °C, 56 °C and 58 °C); Lane 6: No template control (NTC)

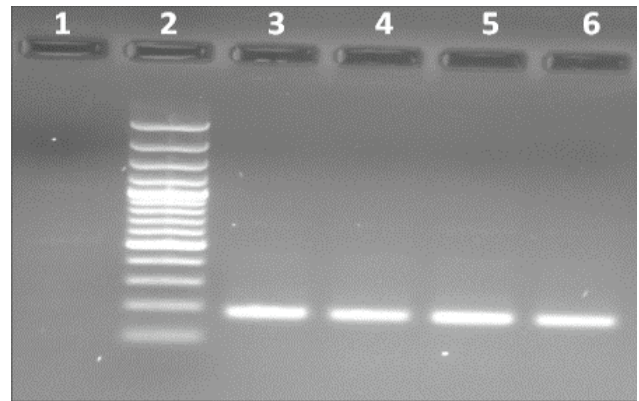


Fig. 2 Gradient PCR using Inner pair of primers
 Lane 1: No template control (NTC); Lane 2: 100 bp Ladder; Lane 3-6: Gradient temperatures (52°C, 54 °C, 56 °C and 58 °C)

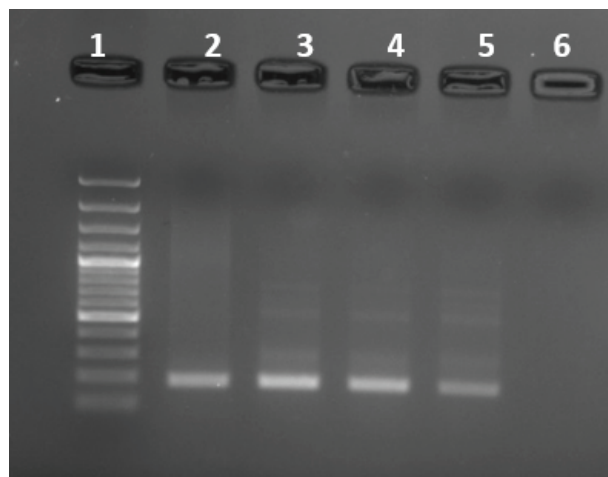


Fig. 3 Gradient PCR using Outer and Inner pair of primers
 Lane 1: 100 bp Ladder; Lane 2-5: Gradient temperatures (52°C, 54 °C, 56 °C and 58°C); Lane 6: NTC

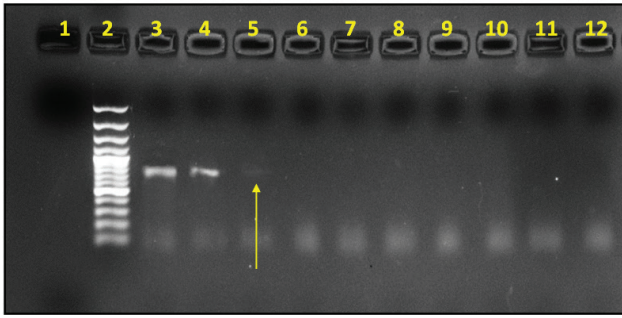


Fig. 4 (a) Sensitivity of Outer primers of nested PCR

Lane 1: NTC; Lane 2: 100 bp DNA Ladder; Lane 3: 2254 ng/ul; Lane 4: 225.2 ng/ul; Lane 5: 22.5 ng/ul; Lane 6: 2.25 ng/ul; Lane 7: 225 pg/ul; Lane 8: 22.5 pg/ul; Lane 9: 2.25 pg/ul; Lane 10: 225 fg/ul; Lane 11: 22.5 fg/ul; Lane 12: 2.2 fg/ul

Amplification was observed up to lane 4 i.e., 22.5 ng/ul of genomic DNA template

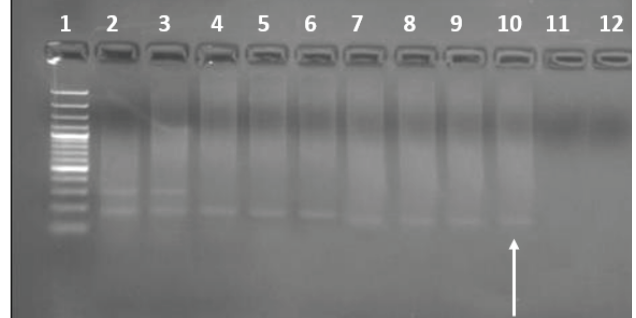


Fig. 4 (b) Sensitivity of Inner primers of Nested PCR

Lane 1: 100 bp DNA Ladder; Lane 2: 2254 ng/ul; Lane 3: 225.2 ng/ul; Lane 4: 22.5 ng/ul; Lane 5: 2.25 ng/ul; Lane 6: 225 pg/ul; Lane 7: 22.5 pg/ul; Lane 8: 2.25 pg/ul; Lane 9: 225 fg/ul; Lane 10: 22.5 fg/ul; Lane 11: 2.2 fg/ul; Lane 12: NTC

Amplification was observed up to lane 10 i.e., 225 fg/ul of genomic DNA template

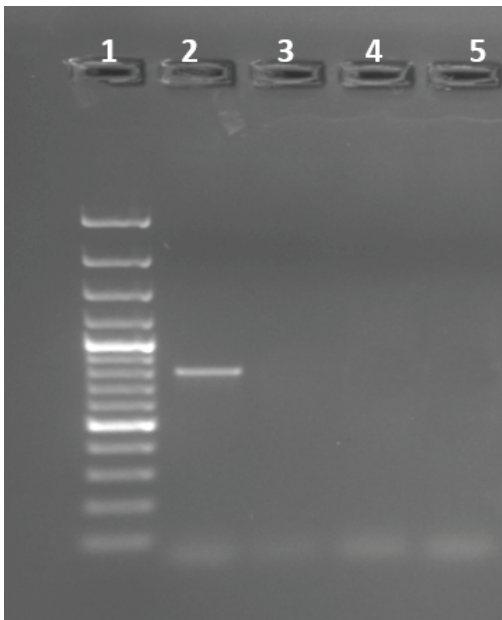


Fig. 5 (a) Specificity Assay using outer primers

Lane 1: 100 bp Ladder; Lane 2: Positive Sample outer amplicon; Lane 3: Bovine Rotavirus; Lane 4: Porcine Kobuvirus; Lane 5: NTC

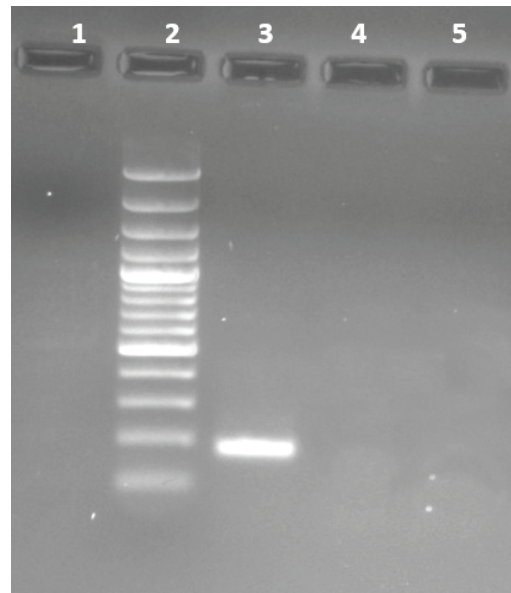


Fig. 5 (b) Specificity Assay using inner primers

Lane 1: 100 bp Ladder; Lane 2: Positive Sample outer amplicon; Lane 3: Bovine Rotavirus; Lane 4: Porcine Kobuvirus; Lane 5: NTC

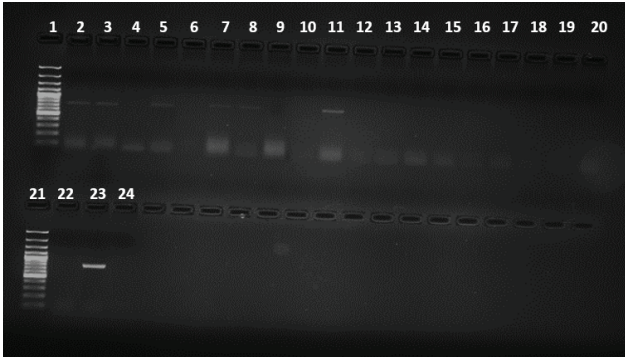


Fig. 6 (a) Assessment of applicability of conventional PCR using outer primers

Lane 1: 100 bp ladder; Lane 2 to Lane 20: P1 to P19 field samples; Lane 21: 100 bp ladder; Lane 22: P20 field sample; Lane 23: Positive Control; Lane 24: NTC

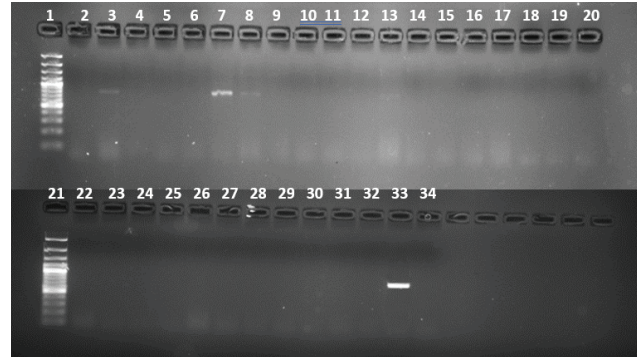


Fig. 6 (b) Assessment of applicability of conventional PCR using outer primers

Lane 1: 100 bp ladder; Lane 2- Lane 20: P21 to P39; Lane 21: 100bp ladder; Lane 22- Lane 32: P 40to P50 field samples; Lane 33: Positive Control; Lane 35: NTC

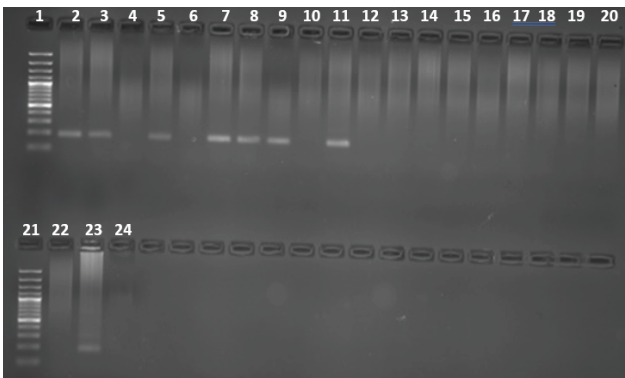


Fig. 7 (a) Assessment of applicability of nPCR. (a) Assessment of applicability of conventional PCR using inner primers

Lane 1: 100 bp ladder; Lane 2 to Lane 20: P1 to P19 field samples; Lane 21: 100 bp ladder; Lane 22: P20 field sample; Lane 23: Positive Control; Lane 24: NTC

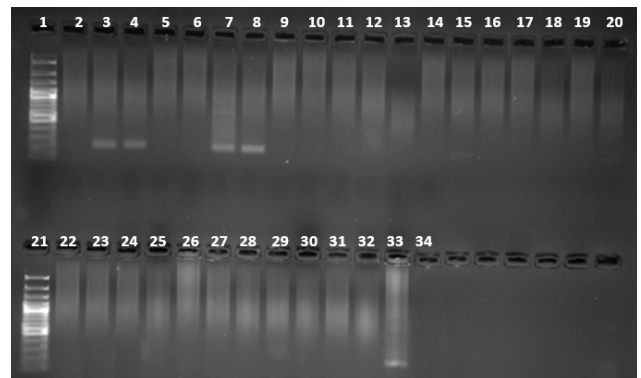


Fig. 7 (b) Assessment of applicability of nPCR. (a) Assessment of applicability of conventional PCR using inner primers

Lane 1: 100 bp ladder; Lane 2- Lane 20: P21 to P39; Lane 21: 100bp ladder; Lane 22- Lane 32: P 40to P50 field samples; Lane 33: Positive Control; Lane 34: NTC