The present study appears to be first of its kind in India to standardize real-time TaqMan PCR for rapid, specific and accurate detection of MG infection in poultry and its comparison with conventional PCR using lipoprotein and 16 S rRNA genes of MG, respectively. Real-time PCR has distinct advantages over conventional PCR, such as higher reliability, rapidity and prevention of false positive result due to environmental contamination during post-amplification analysis (Sprygin et al., 2010). Previous studies reported the detection limits in colony-forming units (CFU), copy number and color changing-units (CCU) (Carli and Eyigor, 2003; Mekkes and Feberwee, 2005; Callison et al., 2006; Jarquin et al., 2009; Raviv and Kleven, 2009; Sprygin et al., 2010). PCR-based methods have replaced culture method for the rapid and accurate detection of Mycoplasma species (Marois et al., 2002; Mekkes and Feberwee, 2005; Fraga et al., 2013; Khalifa et al., 2013; Fujisawa et al., 2019; Yadav et al., 2022b).