**Abstract**

**Background**: Laboratory test results are the cornerstone for patient diagnosis and treatment. Gram staining is a classic laboratory test method used to differentiate between bacteria. Competence assessment can help identify gaps and provide suggestions to academics, researchers, and policymakers to address competency gaps. In Ethiopia, there is no evidence of competency assessment by medical laboratory professionals using the Gram-staining technique.

**Objective**: To assess the competency of medical laboratory professionals on gram stain examination and interpretation in selected hospitals of Addis Ababa, Ethiopia

**Methods**: A cross-sectional study was conducted to assess competency of medical laboratory professionals on gram stain examination and interpretation from September 2015 to December 2017.

**Results:** Of 190 participants, 55(28.9%) participants scored low knowledge, 131(68.9%) scored medium knowledge, and only 4(2.1%) respondents scored high knowledge. From study variables education level, supervision by regional or federal government bodies, and training about Gram staining were significantly associated with the knowledge level of study participants. Forty eight (25.3%), 78(41%), and 64(33.7%) participants scored low, medium, and high skill level respectively from a total of 190 participants. From skill level analysis hospital type, microscope type, and availability of health information resources were significantly associated with skill levels. There were 44 observations (4%) with major errors and 321 observations (28%) with very major errors from all 1140 observations. Of all observations, 321(28.2%) reported without grading, 39 observations (3.4%) reported gram positive bacteria as gram negative bacteria, and 15 observations (1.4%) reported gram negative bacteria as gram positive bacteria.

**Conclusion:** The current study found thatmost medical laboratory professionals work without supervision or refresher training in Gram stain examination and interpretation. Hence, medical laboratory professionals’ knowledge and skill levels are unsatisfactory. Regular competence assessments, training, and follow-up are necessary to improve the professional competence in medical laboratories.