Validation Studies on EZ-PCR Mycoplasma Test Kit (with internal control)

1. **Principle of the EZ PCR Mycoplasma Test Kit**
   The Mycoplasma Detection Kit employs a nucleic acid amplification test on the basis of a polymerase chain reaction. The PCR method allows a fast and highly sensitive detection of mycoplasma contamination in biological samples. The contained primers are specific to a segment of the 16S rRNA region of the mycoplasma genome. The amplified PCR product has a size of approximately 270bp and can directly be made visible in an agarose gel. The selected template is highly conserved within the species Mycoplasma.

   The kit contains positive control (DNA template) and internal control (primers and DNA template) to exclude the possibility for PCR inhibition by the test sample (false negative).

2. The PCR procedure for mycoplasma detection is well documented in the literature and the test is accepted as a very specific and sensitive (with the appropriate primers).

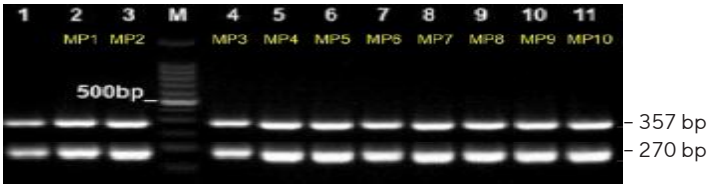
3. The primers developed were tested for years in comparison to the culture detection method and found to be very reliable and specific to mycoplasma (do not react with mammalian or bacterial cells). The primers have broad detection range - more than 95% of the mycoplasma species found in cell culture (Prof. Rotem, Mycoplasma laboratory, Hebrew University Medical School).

Bacterial strains which were tested negative:
E. coli, Entrobacter aerogenes, Bacillus cereus, Streptococcus pyogenes, Proteus, Klebsiella pneumonia, Entrococcus faecalis and staphylococcus aureus.

The mycoplasma species that tested and can be detected by the kit:
Most prevalent in contaminated cell culture: M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. bovis, M. pneumoniae, M. pirum and M. capricolum, as well as Acholeplasma and Spiroplasma species.

In addition, the primers were aligned with the National Center for Biotechnology (NCBI) data base and inspected for homologies within the target region of the 16S rRNA. List of mycoplasma species with relevant sequence homologies is available and include more than 90 species.

With internal control



Positive: 270 bp, Internal control: 357 bp

1	Positive control
MP1	M. Pneumonia
MP2	M. hyorhinis
MP3	M. gallisepticum
MP4	M. fermentans
MP5	M. salivarium
MP6	M. laidlawi
MP7	M. orale
MP8	M. arginini
MP9	M. synoviae
MP10	M. hominis

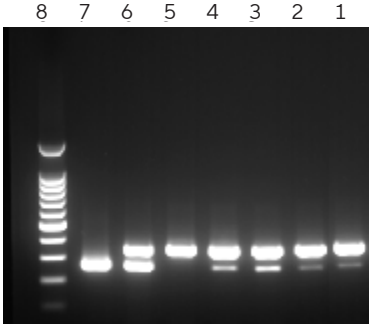
4. The sensitivity of the kit was first tested for the following strains. The mycoplasma concentration wasdetermined by a series of decimal dilutions and culture on agar plates. Test was done after 20x concentration of the test samples as directed in the IFU.

   M. fermentans- 12CFU/ml
   M. capricolum - 5.5CFU/ml
   M. penetrans - 10.0CFU/ml
   M. hyorinis - 10.5CFU/ml
5. 10CFU sensitivity assay: validation of sensitivity of EZ-PCR Mycoplasma Test kit was done using

   10CFU sensitivity standard of the following species:

   Mycoplasma arginini
   Mycoplasma orale
   Mycoplasma gallisepticum
   Mycoplasma pneumoniae
   Mycoplasma synoviae
   Mycoplasma fermentans
   Mycoplasma hyorhinis
   Acholeplasma laidlawii

Test procedure was as instructed in the IFU (20x concentration of the test sample). Positive results were confirmed with and without the internal control supply as part of the kit.



1. Mycoplasma synoviae with IC
2. Mycoplasma fermentans with IC
3. Mycoplasma hyorhinis with IC
4. Acholeplasma laidlawii with IC
5. Negative control
6. DNA controls
7. Positive control
8. Size marker

Positive: 270 bp, Internal control: 357 bp

6. Stability: the original kit was found to be stable for at least 3 years. Positive results obtained with the positive template control and with test sample (mycoplasma contaminated vero cells).

7. The kit is intended for research use only (detection of mycoplasma contamination in cell culture).

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