**APPENDIX A. Supplementary data**

For statistical analysis (Conover, 1999), we used the SAS System for Windows (Statistical Analysis System), version 9.4. SAS Institute Inc, 2002-2008, Cary, NC, USA.

**Table A.1-** Statistical analysis of data obtained from the staining intensity classification of fecal smears in the Experiment I (Mann-Whitney e Kruskal-Wallis).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Experimental protocol** | **Variable** | **N** | **Mean** | **Median** | **Standard deviation** | **Min** | **Max** | **P-value** |
| 1 - Trichrome Modified by Melvin and Brooke  | time5\_min\_tube | 3 | 6,33 | 7,00 | 1,15 | 5,00 | 7,00 | 0,3017\* |
| time10\_min\_tube | 3 | 7,67 | 7,00 | 1,15 | 7,00 | 9,00 | 0,1787 |
| temper35\_tube | 3 | 7,00 | 7,00 | 0,00 | 7,00 | 7,00 | 1,000\*\* |
| temper45\_tube | 3 | 7,00 | 6,00 | 1,73 | 6,00 | 9,00 | 0,7258 |
| conc0.25\_tube | 3 | 8,00 | 8,00 | 0,00 | 8,00 | 8,00 | 0,0755\*\* |
| conc0.50\_tube | 3 | 6,67 | 6,00 | 1,15 | 6,00 | 8,00 | 0,8137\*\*\* |
| pHalk\_tube | 3 | 5,33 | 5,00 | 0,58 | 5,00 | 6,00 | 1,000 |
| phacid\_tube | 3 | 8,00 | 8,00 | 1,00 | 7,00 | 9,00 | 0,5826 |
| time5\_min\_slide | 3 | 7,33 | 7,00 | 0,58 | 7,00 | 8,00 | 0,8465 |
| time10\_min\_slide | 3 | 6,00 | 6,00 | 1,00 | 5,00 | 7,00 | 0,2069 |
| temper35\_slide | 3 | 6,33 | 6,00 | 1,53 | 5,00 | 8,00 | 0,097 |
| temper45\_slide | 3 | 7,33 | 7,00 | 0,58 | 7,00 | 8,00 | 0,0558 |
| conc0.25\_slide | 3 | 7,67 | 8,00 | 0,58 | 7,00 | 8,00 | − |
| conc0.50\_slide | 3 | 6,67 | 6,00 | 1,15 | 6,00 | 8,00 | 0,6428\*\*\* |
| pHalk\_slide | 3 | 5,33 | 5,00 | 0,58 | 5,00 | 6,00 | − |
| phacid\_slide | 3 | 8,00 | 8,00 | 1,00 | 7,00 | 9,00 | 0,8137\*\*\* |
| 2- Trichrome Modified by Wheatley  | time5\_min\_tube | 3 | 8,00 | 8,00 | 0,00 | 8,00 | 8,00 |  |
| time10\_min\_tube | 3 | 6,33 | 6,00 | 0,58 | 6,00 | 7,00 |  |
| temper35\_tube | 3 | 5,00 | 5,00 | 1,00 | 4,00 | 6,00 |  |
| temper45\_tube | 3 | 6,00 | 5,00 | 1,73 | 5,00 | 8,00 |  |
| conc0.25\_tube | 3 | 8,33 | 8,00 | 0,58 | 8,00 | 9,00 |  |
| conc0.50\_tube | 3 | 6,00 | 6,00 | 2,00 | 4,00 | 8,00 |  |
| pHalk\_tube | 3 | 8,00 | 8,00 | 0,00 | 8,00 | 8,00 |  |
| phacid\_tube | 3 | 7,33 | 7,00 | 0,58 | 7,00 | 8,00 |  |
| time5\_min\_slide | 3 | 7,00 | 7,00 | 1,00 | 6,00 | 8,00 |  |
| time10\_min\_slide | 3 | 7,67 | 7,00 | 1,15 | 7,00 | 9,00 |  |
| temper35\_slide | 3 | 4,33 | 4,00 | 0,58 | 4,00 | 5,00 |  |
| temper45\_slide | 3 | 4,67 | 4,00 | 1,15 | 4,00 | 6,00 |  |
| conc0.25\_slide | 3 | 7,00 | 7,00 | 0,00 | 7,00 | 7,00 |  |
| conc0.50\_slide | 3 | 5,67 | 6,00 | 1,53 | 4,00 | 7,00 |  |
| pHalk\_slide | 3 | 8,00 | 8,00 | 0,00 | 8,00 | 8,00 |  |
| phacid\_slide | 3 | 7,67 | 8,00 | 0,58 | 7,00 | 8,00 |  |
| 3- Weber’s green stain for detection of microsporidia  | time5\_min\_tube | 3 | 7,33 | 7,00 | 0,58 | 7,00 | 8,00 |  |
| time10\_min\_tube | 3 | 6,67 | 7,00 | 0,58 | 6,00 | 7,00 |  |
| temper35\_tube | 3 | 5,00 | 5,00 | 1,00 | 4,00 | 6,00 |  |
| temper45\_tube | 3 | 6,00 | 7,00 | 1,73 | 4,00 | 7,00 |  |
| conc0.25\_tube | 3 | 6,00 | 6,00 | 1,00 | 5,00 | 7,00 |  |
| conc0.50\_tube | 3 | 4,00 | 4,00 | 0,00 | 4,00 | 4,00 |  |
| pHalk\_tube | 3 | 5,67 | 5,00 | 1,15 | 5,00 | 7,00 |  |
| phacid\_tube | 3 | 7,67 | 8,00 | 0,58 | 7,00 | 8,00 |  |
| time5\_min\_slide | 3 | 7,33 | 7,00 | 0,58 | 7,00 | 8,00 |  |
| time10\_min\_slide | 3 | 6,33 | 7,00 | 1,15 | 5,00 | 7,00 |  |
| temper35\_slide | 3 | 5,33 | 5,00 | 0,58 | 5,00 | 6,00 |  |
| temper45\_slide | 3 | 5,00 | 5,00 | 1,00 | 4,00 | 6,00 |  |
| conc0.25\_slide | 3 | 4,00 | 4,00 | 0,00 | 4,00 | 4,00 |  |
| conc0.50\_slide | 3 | 4,00 | 4,00 | 0,00 | 4,00 | 4,00 |  |
| pHalk\_slide | 3 | 4,00 | 4,00 | 0,00 | 4,00 | 4,00 |  |
| phacid\_slide | 3 | 4,00 | 4,00 | 0,00 | 4,00 | 4,00 |  |
| \* protocol 2 not evaluated; \*\*protocol 1 not evaluated; \*\*\*protocol 3 not evaluated. |  |  |  |  |  |

**Table A.2-** Statistical analysis of data obtained from the staining intensity classification of fecal smears in the Experiment II (Mann-Whitney e Kruskal-Wallis).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Experimental protocol** | **Variable** | **N** | **Mean** | **Median** | **Standard deviation** | **Min** | **Max** | **P-value** |
| 1-Indigotine Blue | av1\*\_tube | 3 | 7,33 | 8 | 1,15 | 6 | 8 | **0,0269\*\*\*** |
| av1\_slide | 3 | 6,67 | 7 | 0,58 | 6 | 7 | 0,1239 |
| av2\*\*\_tube | 3 | 6 | 6 | 1 | 5 | 7 | 0,6531 |
| av2\_slide | 3 | 5 | 5 | 0 | 5 | 5 |  |
| 2- Methylene Blue | av1\_tube | 3 | 0 | 0 | 0 | 0 | 0 |  |
| av1\_slide | 3 | 0 | 0 | 0 | 0 | 0 |  |
| av2\_tube | 3 | 5,33 | 5 | 1,53 | 4 | 7 |  |
| av2\_slide | 3 | 0 | 0 | 0 | 0 | 0 |  |
| 3- Methylene Blue 0,20% | av1\_tube | 3 | 4 | 4 | 0 | 4 | 4 |  |
| av1\_slide | 3 | 4 | 4 | 0 | 4 | 4 |  |
| av2\_tube | 3 | 0 | 0 | 0 | 0 | 0 |  |
| av2\_slide | 3 | 4 | 4 | 0 | 4 | 4 |  |
| 4- Acetic Orcein | av1\_tube | 3 | 6,33 | 6 | 1,53 | 5 | 8 |  |
| av1\_slide | 3 | 7 | 6 | 1,73 | 6 | 9 |  |
| av2\_tube | 3 | 5 | 5 | 0 | 5 | 5 |  |
| av2\_slide | 3 | 5 | 5 | 0 | 5 | 5 |  |
| 5- Gentian Violet 0,5% | av1\_tube | 3 | 3,33 | 3 | 0,58 | 3 | 4 |  |
| av1\_slide | 3 | 3 | 3 | 0 | 3 | 3 |  |
| av2\_tube | 3 | 0 | 0 | 0 | 0 | 0 |  |
| av2\_slide | 3 | 0 | 0 | 0 | 0 | 0 |  |
| 6- Gentian Violet 0,25% | av1\_tube | 3 | 4 | 4 | 0 | 4 | 4 |  |
| av1\_slide | 3 | 3,33 | 3 | 0,58 | 3 | 4 |  |
| av2\_tube | 3 | 0 | 0 | 0 | 0 | 0 |  |
| av2\_slide | 3 | 0 | 0 | 0 | 0 | 0 |  |
| 7- Gentian Violet – 0,1% | av1\_tube | 3 | 4,33 | 4 | 0,58 | 4 | 5 |  |
| av1\_slide | 3 | 4 | 4 | 0 | 4 | 4 |  |
| av2\_tube | 3 | 0 | 0 | 0 | 0 | 0 |  |
| av2\_slide | 3 | 4,67 | 5 | 0,58 | 4 | 5 |  |
| \*av1 = experimental dye + trichrome solution; \*\*av2 = experimental dye + D´Antoni's iodine solution: \*\*\*(difference: 1 > 5 and; 4 > 7) |  |

**Table A.3**. Reduction in the number of candidates obtained and the respective sensitivity in each stage of the digital treatment of images containing *Cryptosporidium* spp. oocysts.

|  |  |  |
| --- | --- | --- |
| **Step** | **Sensitivity** | **Number of candidate patches** |
| Segmentation in superpixels | 100% | 10309.5 ± 18.4 |
| Clustering of patches | 100% | 1000 ± 0 |
| Filtering by size | 98% | 266.2 ± 69.5 |

**References of Supplementary data**

Conover, W. J. (1999). *Practical nonparametric statistics*. 3rd ed. New York: Wiley.