

DERMAKIT

SUITABILITY STUDY

This study was performed to evaluate the capacity for growth and selectivity of the DSM culture medium.

This study was carried out to evaluate the capacity over a period of time of the DSM culture medium used in the DERMAKIT kit.

The DSM taken from two Group A1 (for 36 months) and Group A2 (for 24 months), was experimentally inoculated with strains of different dermatophytes, saprophytic mycetes and bacteria strains including *Aspergillus fumigatus*, *Candida albicans* and *Escherichia coli*, in order to show, at an experimental level, the degree of specificity of the test.

Batch A1 : Batch n. 0015 – 1000 vials were stored at refrigerated temperature and controls were carried out every month for 36 months.

Batch A2 : Batch n. 0015 – 1000 vials were stored at room temperature and controls were carried out every month for 24 months.

The microorganism strains used for inoculation are reported below:

| | |
|----|------------------------------------|
| MC | <i>Microsporum canis</i> |
| MG | <i>Microsporum gypseum</i> |
| TE | <i>Trichophyton equinum</i> |
| TM | <i>Trichophyton mentagrophytes</i> |
| TT | <i>Trichophyton tonsurans</i> |
| TR | <i>Trichophyton rubrum</i> |
| EF | <i>Epidermophyton floccosum</i> |
| AF | <i>Aspergillus fumigatus</i> |
| CA | <i>Candida albicans</i> |
| EC | <i>Escherichia coli</i> |



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After inoculation, the cap was put on the vial (but not screwed tightly), so as to allow air to circulate freely. The vial was then incubated at room temperature for 14 days.

At different hour intervals, and, after the 84th hour, every 24 hours for 14 days, the culture medium was checked for any colour change (yellow ochre to red) and the growth of colonies.

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1.1 Suitability under refrigerated storage

The vials of the DERMAKIT diagnostic kit from Batch A1 were inoculated in quadruplicate (each sample), over a period of 36 months (2, 184, 366, 577, 759, 941 and 1095 days) after the preparation of the culture medium.

| | |
|---------------------------------|-------------------------|
| Theoretical expiry date: | 36 months (3 years) |
| End of the study: | (36 months = 1095 days) |
| Store temperature: | + 4°C +/- 3°C |

Results:

| | |
|--------------------------------|----------------|
| Total samples tested: | 490 |
| Positive samples: | 205/205 (100%) |
| Negative samples: | 285/285 (100%) |
| False negatives: | 0 (0%) |
| False positives: | 0 (0%) |
| Correct identification: | 490 (100%) |
| Specificity: | 100% |
| Sensitivity: | 100% |

| | |
|--|--------|
| Mean of colour change in hours: | 30 |
| Median of colour change in hours: | 31 |
| Standard deviation: | 4.1070 |
| Coefficient of variation: | 13.52% |

| | |
|----------------------------------|--------|
| Growth mean in hours: | 73 |
| Growth median in hours: | 74 |
| Standard deviation: | 3.0412 |
| Coefficient of variation: | 4.16% |

Table 2.1.1: statistical data regarding Batch A1 vials inoculated with different strains and evaluated over a period of time of 36 months (stored at refrigerated temperature).

| Period | Samp. | Strain | Colour change hours | Mean | SD | CV% | Growth hours | Mean | SD | CV% |
|--------|-------|--------|---------------------|------|------|--------|--------------|------|------|-------|
| 2 | 10 | MC | YES | 25 | 2,45 | 9,80% | YES | 77 | 1,45 | 1,88% |
| 184 | 10 | MC | YES | 25 | 2,08 | 8,32% | YES | 74 | 2,59 | 3,50% |
| 366 | 10 | MC | YES | 30 | 2,31 | 7,70% | YES | 76 | 1,55 | 2,04% |
| 577 | 10 | MC | YES | 26 | 2,26 | 8,69% | YES | 72 | 1,58 | 2,19% |
| 759 | 10 | MC | YES | 31 | 2,95 | 9,52% | YES | 72 | 1,52 | 2,11% |
| 941 | 10 | MC | YES | 31 | 2,78 | 8,97% | YES | 72 | 2,89 | 4,01% |
| 1095 | 10 | MC | YES | 30 | 2,65 | 8,83% | YES | 74 | 2,74 | 3,70% |
| | | | | | | | | | | |
| 2 | 10 | MG | YES | 30 | 3,12 | 10,40% | YES | 70 | 2,63 | 3,76% |
| 184 | 10 | MG | YES | 29 | 2,56 | 8,83% | YES | 72 | 1,52 | 2,11% |
| 366 | 10 | MG | YES | 31 | 2,95 | 9,52% | YES | 74 | 2,08 | 2,81% |
| 577 | 10 | MG | YES | 27 | 2,57 | 9,52% | YES | 74 | 2,95 | 3,99% |
| 759 | 10 | MG | YES | 31 | 2,68 | 8,65% | YES | 74 | 1,85 | 2,50% |
| 941 | 10 | MG | YES | 31 | 2,89 | 9,32% | YES | 78 | 1,46 | 1,87% |
| 1095 | 10 | MG | YES | 32 | 3,66 | 11,44% | YES | 74 | 2,55 | 3,45% |
| | | | | | | | | | | |
| 2 | 10 | TE | YES | 31 | 2,00 | 6,45% | YES | 68 | 2,75 | 4,04% |
| 184 | 10 | TE | YES | 49 | 2,45 | 5,00% | YES | 72 | 1,99 | 2,76% |
| 366 | 10 | TE | YES | 31 | 2,18 | 7,03% | YES | 72 | 2,55 | 3,54% |
| 577 | 10 | TE | YES | 31 | 2,35 | 7,58% | YES | 76 | 2,84 | 3,74% |
| 759 | 10 | TE | YES | 28 | 2,48 | 8,86% | YES | 72 | 2,36 | 3,28% |
| 941 | 10 | TE | YES | 30 | 3,08 | 10,27% | YES | 68 | 2,19 | 3,22% |
| 1095 | 10 | TE | YES | 32 | 3,06 | 9,56% | YES | 72 | 2,87 | 3,99% |
| | | | | | | | | | | |
| 2 | 10 | TM | YES | 26 | 2,31 | 8,88% | YES | 68 | 2,54 | 3,74% |
| 184 | 10 | TM | YES | 32 | 2,00 | 6,25% | YES | 68 | 1,69 | 2,49% |
| 366 | 10 | TM | YES | 29 | 2,56 | 8,83% | YES | 70 | 1,36 | 1,94% |
| 577 | 10 | TM | YES | 25 | 2,56 | 10,24% | YES | 70 | 3,08 | 4,40% |
| 759 | 10 | TM | YES | 31 | 2,85 | 9,19% | YES | 72 | 2,12 | 2,94% |
| 941 | 10 | TM | YES | 26 | 2,31 | 8,88% | YES | 72 | 2,05 | 2,85% |
| 1095 | 10 | TM | YES | 26 | 2,36 | 9,08% | YES | 74 | 2,58 | 3,49% |
| | | | | | | | | | | |
| 2 | 10 | TT | YES | 30 | 2,56 | 8,53% | YES | 74 | 2,14 | 2,89% |
| 184 | 10 | TT | YES | 24 | 2,00 | 8,33% | YES | 78 | 3,09 | 3,96% |
| 366 | 10 | TT | YES | 32 | 2,00 | 6,25% | YES | 74 | 3,12 | 4,22% |
| 577 | 10 | TT | YES | 32 | 2,05 | 6,41% | YES | 74 | 2,56 | 3,46% |
| 759 | 10 | TT | YES | 28 | 2,56 | 9,14% | YES | 72 | 2,43 | 3,38% |
| 941 | 10 | TT | YES | 26 | 2,66 | 10,23% | YES | 72 | 2,36 | 3,28% |
| 1095 | 10 | TT | YES | 25 | 2,46 | 9,84% | YES | 74 | 2,85 | 3,85% |

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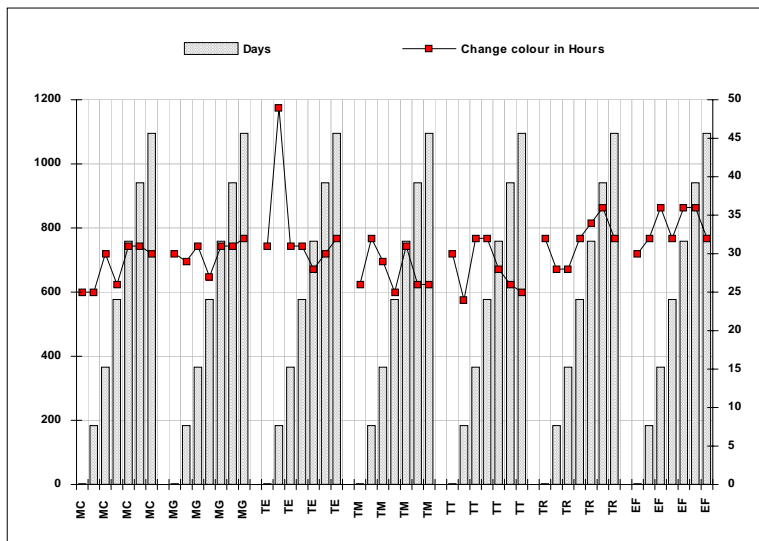
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| Period | Samp. | Strain | Colour change hours | Mean | SD | CV% | Growth hours | Mean | SD | CV% |
|--------|-------|--------|---------------------|------|------|--------|--------------|------|------|-------|
| 2 | 10 | TR | YES | 32 | 2,56 | 8,00% | YES | 68 | 1,56 | 2,29% |
| 184 | 10 | TR | YES | 28 | 2,41 | 8,61% | YES | 70 | 2,45 | 3,50% |
| 366 | 10 | TR | YES | 28 | 2,00 | 7,14% | YES | 74 | 3,66 | 4,95% |
| 577 | 10 | TR | YES | 32 | 2,15 | 6,72% | YES | 78 | 1,56 | 2,00% |
| 759 | 10 | TR | YES | 34 | 2,15 | 6,32% | YES | 74 | 2,54 | 3,43% |
| 941 | 10 | TR | YES | 36 | 2,00 | 5,56% | YES | 74 | 2,56 | 3,46% |
| 1095 | 10 | TR | YES | 32 | 2,00 | 6,25% | YES | 76 | 2,66 | 3,50% |
| | | | | | | | | | | |
| 2 | 10 | EF | YES | 30 | 3,15 | 10,50% | YES | 68 | 3,11 | 4,57% |
| 184 | 10 | EF | YES | 32 | 2,85 | 8,91% | YES | 68 | 1,52 | 2,24% |
| 366 | 10 | EF | YES | 36 | 2,45 | 6,81% | YES | 68 | 1,64 | 2,41% |
| 577 | 10 | EF | YES | 32 | 2,48 | 7,75% | YES | 70 | 1,61 | 2,30% |
| 759 | 10 | EF | YES | 36 | 2,56 | 7,11% | YES | 70 | 2,61 | 3,73% |
| 941 | 10 | EF | YES | 36 | 2,56 | 7,11% | YES | 70 | 2,42 | 3,46% |
| 1095 | 10 | EF | YES | 32 | 2,55 | 7,97% | YES | 72 | 2,50 | 3,47% |
| | | | | | | | | | | |
| 2 | 10 | AF | NO | -- | -- | -- | YES | 74 | 1,62 | 2,19% |
| 184 | 10 | AF | NO | -- | -- | -- | YES | 74 | 1,24 | 1,68% |
| 366 | 10 | AF | NO | -- | -- | -- | YES | 76 | 3,25 | 4,28% |
| 577 | 10 | AF | NO | -- | -- | -- | YES | 78 | 1,84 | 2,36% |
| 759 | 10 | AF | NO | -- | -- | -- | YES | 78 | 1,78 | 2,28% |
| 941 | 10 | AF | NO | -- | -- | -- | YES | 74 | 3,05 | 4,12% |
| 1095 | 10 | AF | NO | -- | -- | -- | YES | 72 | 1,26 | 1,75% |
| | | | | | | | | | | |
| 2 | 10 | CA | NO | -- | -- | -- | YES | 74 | 1,52 | 2,05% |
| 184 | 10 | CA | NO | -- | -- | -- | YES | 74 | 1,48 | 2,00% |
| 366 | 10 | CA | NO | -- | -- | -- | YES | 74 | 1,54 | 2,08% |
| 577 | 10 | CA | NO | -- | -- | -- | YES | 78 | 1,36 | 1,74% |
| 759 | 10 | CA | NO | -- | -- | -- | YES | 78 | 2,15 | 2,76% |
| 941 | 10 | CA | NO | -- | -- | -- | YES | 78 | 1,09 | 1,40% |
| 1095 | 10 | CA | NO | -- | -- | -- | YES | 78 | 1,22 | 1,56% |
| | | | | | | | | | | |
| 2 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 184 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 366 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 577 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 759 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 941 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 1095 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |

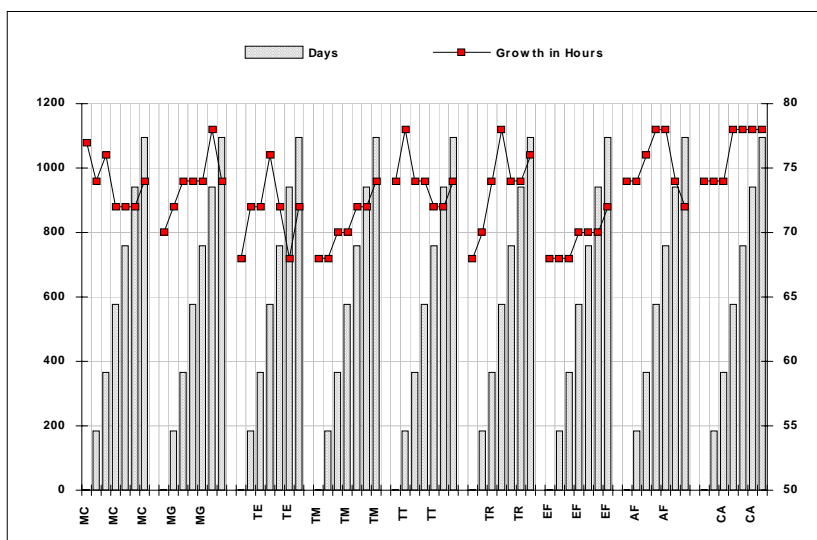
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Graph 2.1.2: evaluation of changing colours in hours relative to Batch A1 vials inoculated with different strains and evaluated over a period of time of 36 months (stored at refrigerated temperature).



Graph 2.1.3: evaluation of growth in hours of different strains inoculated in Group A1 vials evaluated over a period of time of 36 months (stored at refrigerated temperature).



2.2 Suitability under room temperature storage

The vials of the DERMAKIT diagnostic kit from Batch A2, were inoculated in quadruplicate (each sample), over a period of 24 months (2, 184, 366, 577 and 730 days) after the preparation of the culture medium.

| | |
|---------------------------------|----------------------|
| Theoretical expiry date: | 36 months (3 years) |
| End of the study: | 24 months = 730 days |
| Store temperature: | + 18/26 °C |

Results:

| | |
|--|----------------|
| Total samples tested: | 490 |
| Positive samples: | 205/205 (100%) |
| Negative samples: | 285/285 (100%) |
| False negatives: | 0 (0%) |
| False positives: | 0 (0%) |
| Correct identification: | 490 (100%) |
| Specificity: | 100% |
| Sensitivity: | 100% |
| | |
| Mean of colour change in hours: | 30 |
| Median of colour change in hours: | 29 |
| Standard deviation: | 3.5142 |
| Coefficient of variation: | 11.85% |
| | |
| Growth mean in hours: | 71 |
| Growth median in hours: | 72 |
| Standard deviation: | 7.8869 |
| Coefficient of variation: | 11.07% |

Table 2.2.1: statistic data relative to Batch A2 vials inoculated with different strains and evaluated over a period of time of 24 months (stored at room temperature).

| Period | Samp. | Strain | Colour change hours | Mean | SD | CV% | Growth hours | Mean | SD | CV% |
|--------|-------|--------|---------------------|------|-------|--------|--------------|------|-------|--------|
| 2 | 10 | MC | YES | 27 | 1,93 | 7,21% | YES | 66 | 9,66 | 14,64% |
| 184 | 10 | MC | YES | 28 | 2,95 | 10,39% | YES | 62 | 10,19 | 16,33% |
| 366 | 10 | MC | YES | 27 | 3,29 | 12,29% | YES | 60 | 9,13 | 15,12% |
| 577 | 10 | MC | YES | 30 | 2,11 | 7,03% | YES | 65 | 9,81 | 15,04% |
| 730 | 10 | MC | YES | 30 | 2,11 | 7,03% | YES | 68 | 8,73 | 12,77% |
| | | | | | | | | | | |
| 2 | 10 | MG | YES | 33 | 11,47 | 14,05% | YES | 64 | 8,43 | 13,18% |
| 184 | 10 | MG | YES | 38 | 7,77 | 15,13% | YES | 74 | 2,07 | 2,81% |
| 366 | 10 | MG | YES | 28 | 3,27 | 11,66% | YES | 69 | 9,25 | 13,36% |
| 577 | 10 | MG | YES | 30 | 9,66 | 16,32% | YES | 64 | 9,88 | 15,53% |
| 730 | 10 | MG | YES | 29 | 7,32 | 18,12% | YES | 64 | 8,43 | 13,18% |
| | | | | | | | | | | |
| 2 | 10 | TE | YES | 26 | 2,07 | 7,82% | YES | 75 | 3,16 | 4,20% |
| 184 | 10 | TE | YES | 26 | 2,07 | 7,82% | YES | 70 | 7,60 | 10,86% |
| 366 | 10 | TE | YES | 28 | 3,50 | 12,33% | YES | 62 | 10,01 | 16,26% |
| 577 | 10 | TE | YES | 26 | 2,07 | 8,07% | YES | 60 | 9,43 | 15,71% |
| 730 | 10 | TE | YES | 26 | 2,83 | 10,88% | YES | 66 | 8,69 | 13,17% |
| | | | | | | | | | | |
| 2 | 10 | TM | YES | 29 | 3,16 | 10,96% | YES | 68 | 8,73 | 12,77% |
| 184 | 10 | TM | YES | 30 | 2,07 | 6,79% | YES | 78 | 3,37 | 4,35% |
| 366 | 10 | TM | YES | 30 | 2,11 | 7,03% | YES | 65 | 10,46 | 16,15% |
| 577 | 10 | TM | YES | 30 | 2,80 | 9,45% | YES | 74 | 2,80 | 3,76% |
| 730 | 10 | TM | YES | 31 | 1,69 | 5,41% | YES | 75 | 2,53 | 3,36% |
| | | | | | | | | | | |
| 2 | 10 | TT | YES | 38 | 9,28 | 19,50% | YES | 76 | 3,27 | 4,30% |
| 184 | 10 | TT | YES | 29 | 1,93 | 6,62% | YES | 74 | 2,07 | 2,81% |
| 366 | 10 | TT | YES | 30 | 3,37 | 11,40% | YES | 74 | 2,83 | 3,82% |
| 577 | 10 | TT | YES | 28 | 3,27 | 11,66% | YES | 76 | 3,50 | 4,63% |
| 730 | 10 | TT | YES | 28 | 3,50 | 12,69% | YES | 78 | 2,07 | 2,66% |
| | | | | | | | | | | |
| 2 | 10 | TR | YES | 27 | 3,16 | 11,60% | YES | 76 | 3,50 | 4,63% |
| 184 | 10 | TR | YES | 31 | 1,69 | 5,41% | YES | 75 | 3,16 | 4,20% |
| 366 | 10 | TR | YES | 26 | 2,11 | 8,11% | YES | 78 | 2,07 | 2,66% |
| 577 | 10 | TR | YES | 27 | 1,93 | 7,21% | YES | 75 | 3,68 | 4,89% |
| 730 | 10 | TR | YES | 28 | 2,95 | 10,39% | YES | 75 | 2,53 | 3,36% |
| | | | | | | | | | | |
| 2 | 10 | EF | YES | 27 | 3,29 | 12,29% | YES | 73 | 1,69 | 2,32% |
| 184 | 10 | EF | YES | 28 | 3,27 | 11,66% | YES | 75 | 3,16 | 4,20% |

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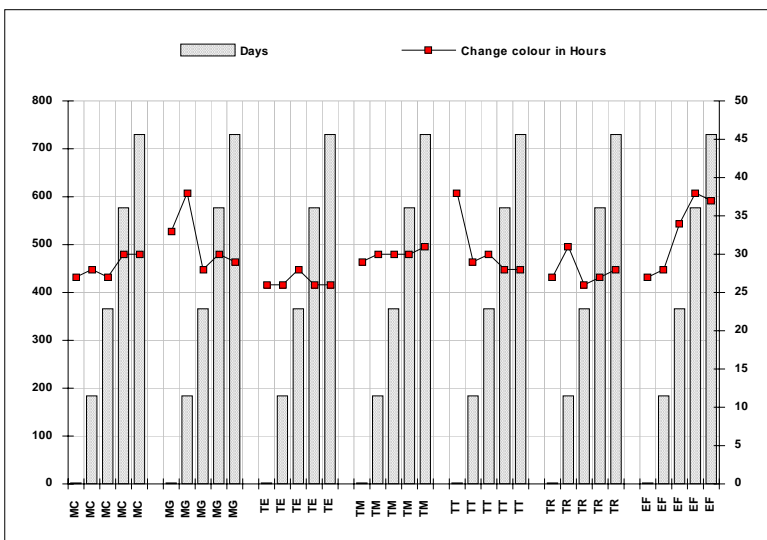
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| Period | Samp. | Strain | Colour change hours | Mean | SD | CV% | Growth hours | Mean | SD | CV% |
|--------|-------|--------|---------------------|------|------|--------|--------------|------|------|--------|
| 366 | 10 | EF | YES | 34 | 7,63 | 18,13% | YES | 76 | 2,95 | 3,86% |
| 577 | 10 | EF | YES | 38 | 7,23 | 18,83% | YES | 76 | 3,27 | 4,30% |
| 730 | 10 | EF | YES | 37 | 6,81 | 18,31% | YES | 73 | 1,69 | 2,32% |
| 2 | 10 | AF | NO | -- | -- | -- | YES | 76 | 2,67 | 3,51% |
| 184 | 10 | AF | NO | -- | -- | -- | YES | 75 | 2,70 | 3,61% |
| 366 | 10 | AF | NO | -- | -- | -- | YES | 74 | 2,07 | 2,81% |
| 577 | 10 | AF | NO | -- | -- | -- | YES | 74 | 2,80 | 3,80% |
| 730 | 10 | AF | NO | -- | -- | -- | YES | 78 | 2,07 | 2,66% |
| | | | | | | | | | | |
| 2 | 10 | CA | NO | -- | -- | -- | YES | 74 | 2,80 | 3,76% |
| 184 | 10 | CA | NO | -- | -- | -- | YES | 75 | 3,29 | 4,40% |
| 366 | 10 | CA | NO | -- | -- | -- | YES | 64 | 8,93 | 13,87% |
| 577 | 10 | CA | NO | -- | -- | -- | YES | 68 | 8,43 | 12,40% |
| 730 | 10 | CA | NO | -- | -- | -- | YES | 70 | 7,35 | 10,56% |
| | | | | | | | | | | |
| 2 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 184 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 366 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 577 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |
| 730 | 10 | EC | NO | -- | -- | -- | NC | -- | -- | -- |

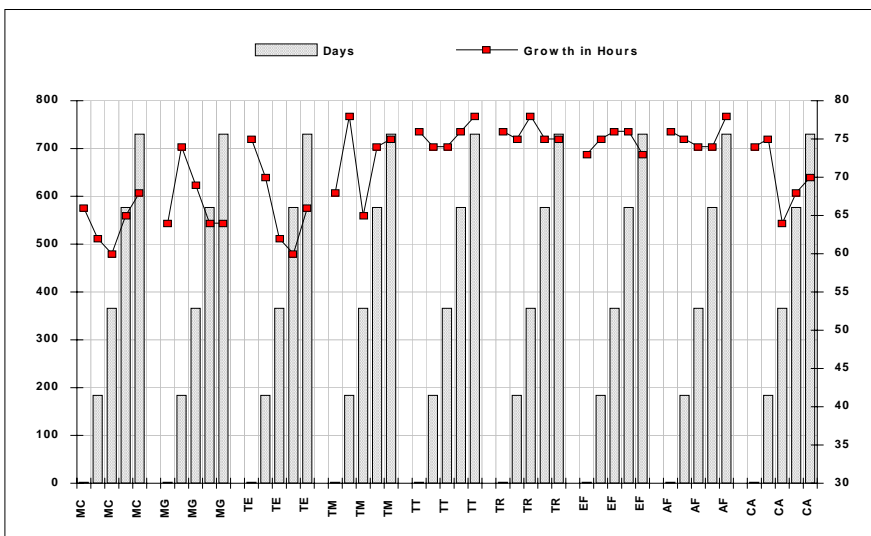
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Graph 2.2.2: evaluation of changing colours in hours regarding Batch A2 vials inoculated with different strains and evaluated over a period of time of 24 months (stored at room temperature).



Graph 2.2.3: evaluation of growth in hours of different strains inoculated in Batch A2 vials evaluated over a period of time of 24 months (stored at room temperature).



CONCLUSIONS

Table 2.1.1 and graphs 2.1.2 and 2.1.3, for Batch A1, show the high degree of specificity of the system, (100%), with the absence of false positives and false negatives results.

Table 2.2.1 and graphs 2.2.2 and 2.2.3, for Batch A2, show the high degree of specificity of the system, (100%), with the absence of false positives and false negatives results.

For Batch A1 once inoculated, the culture mediums were incubated at room temperature.

On average, a colour change from yellow ochre to red was observed within 30 hours with a variation coefficient of 13,52%, and the appearance of colonies (= clear growth) in 73 hours with a variation coefficient of 4,16%.

The same for Batch A2: once inoculated, the culture mediums were incubated at room temperature. On average, a colour change from yellow ochre to red was observed within 30 hours with a variation coefficient of 11,85%, and the appearance of colonies (= clear growth) in 71 hours with a variation coefficient of 11,07%.

Data obtained demonstrate a good repeatability, mainly under variable conditions of incubation (=incubation at room temperature). Identification of the colonies following incubation at room temperature was found to be relatively easy.

This study basically shows the very high specificity of the DSM culture medium of the DERMAKIT test; it remains high even when colour change is used as the only element for positivity. The culture medium demonstrates to be very stable over a period of time (whether refrigerated for 36 months, or kept at room temperature for 24 months). The use of glass vials with a screw-top and a seal, which prevents drying up of the culture medium, contributes to preserving the test's characteristics.

The culture medium shows rapid development of colonies, considering the fact that just room temperature is required (21 - 25 °C), without incubation, thus exposing the medium to a wide range of variations of temperature and humidity. It, therefore, follows that the DERMAKIT test



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represents a valid and rapid method of analysis (24 - 72 hours) for screening for the presence of dermatophyte infections in dogs, cats and horses, in addition to other animals and humans. The test is based on the colour change of the culture medium, allowing the pathogenic agent to be quickly and simply identified, with a very high degree of specificity. It should be stressed that, in the case of slow growing strains of dermatophytes, there is an increase in the response time, which varies from 96 to 120 hours after the inoculation.

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