# DISTRIBUTION UNCERTAINTY
# &
# CALCULATIONS IN EXCEL TOOL

GUNNAR ANDERSSON, SVA

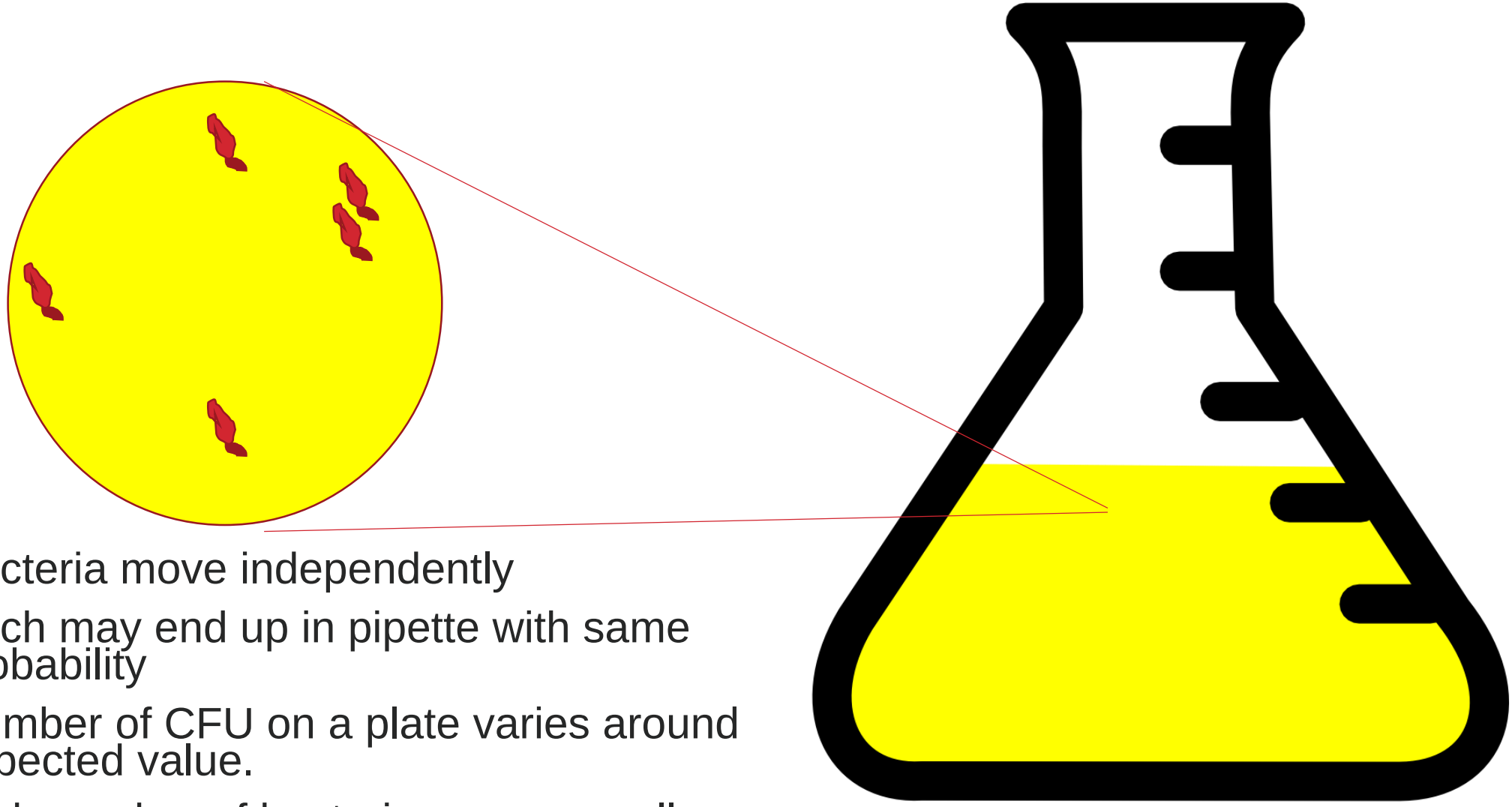
# DISTRIBUTIONAL UNCERTAINTY

- irreducible minimum uncertainty components
  (assuming homogeneous material/perfect mixing)
- specific to individual measurement result
- can be calculated from look up tables (no practical work);
  - ISO 19036 provides details
- Formulas & tables implemented in excel tool

# TYPES OF DISTRIBUTION UNCERTAINTY

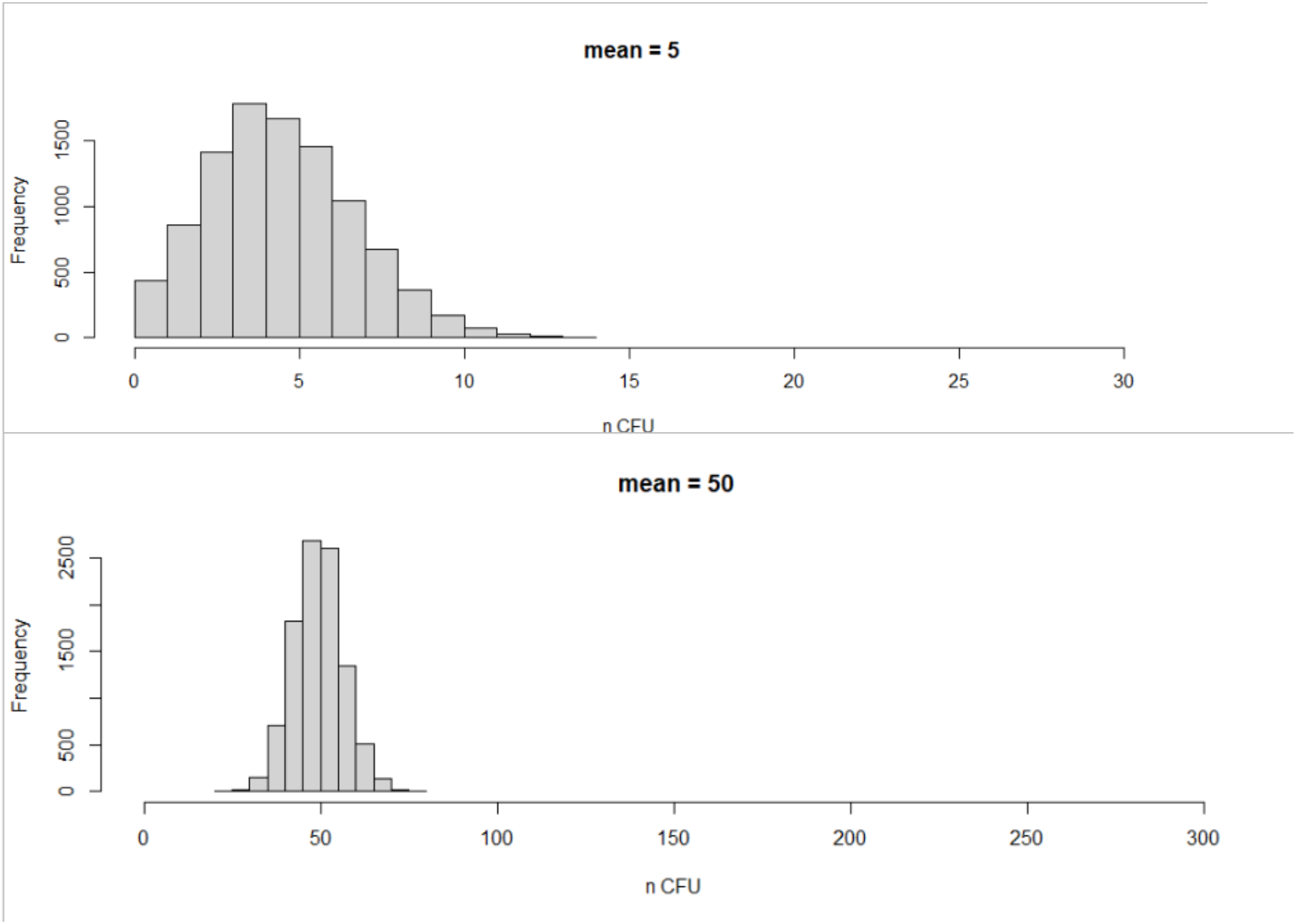
- Poisson uncertainty
- confirmation uncertainty
- Most Probable Number uncertainty

# POISSON UNCERTAINTY



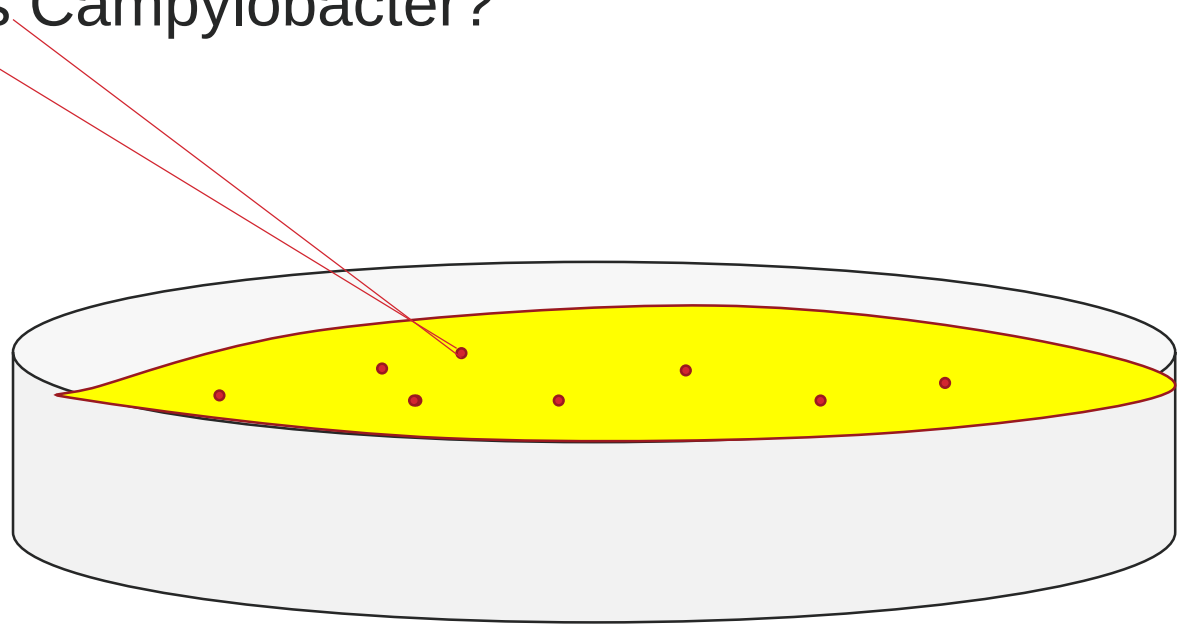
- Bacteria move independently
- Each may end up in pipette with same probability
- Number of CFU on a plate varies around expected value.
- High number of bacteria means smaller relative uncertainty

# POISSON UNCERTAINTY



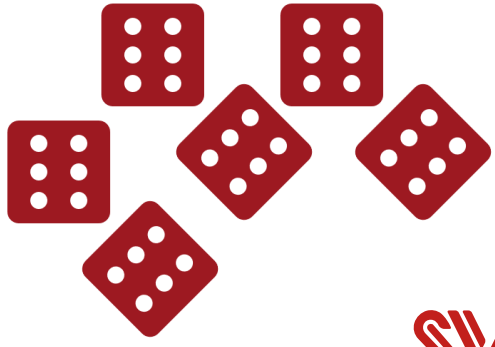
# CONFIRMATION UNCERTAINTY

Is this Campylobacter?

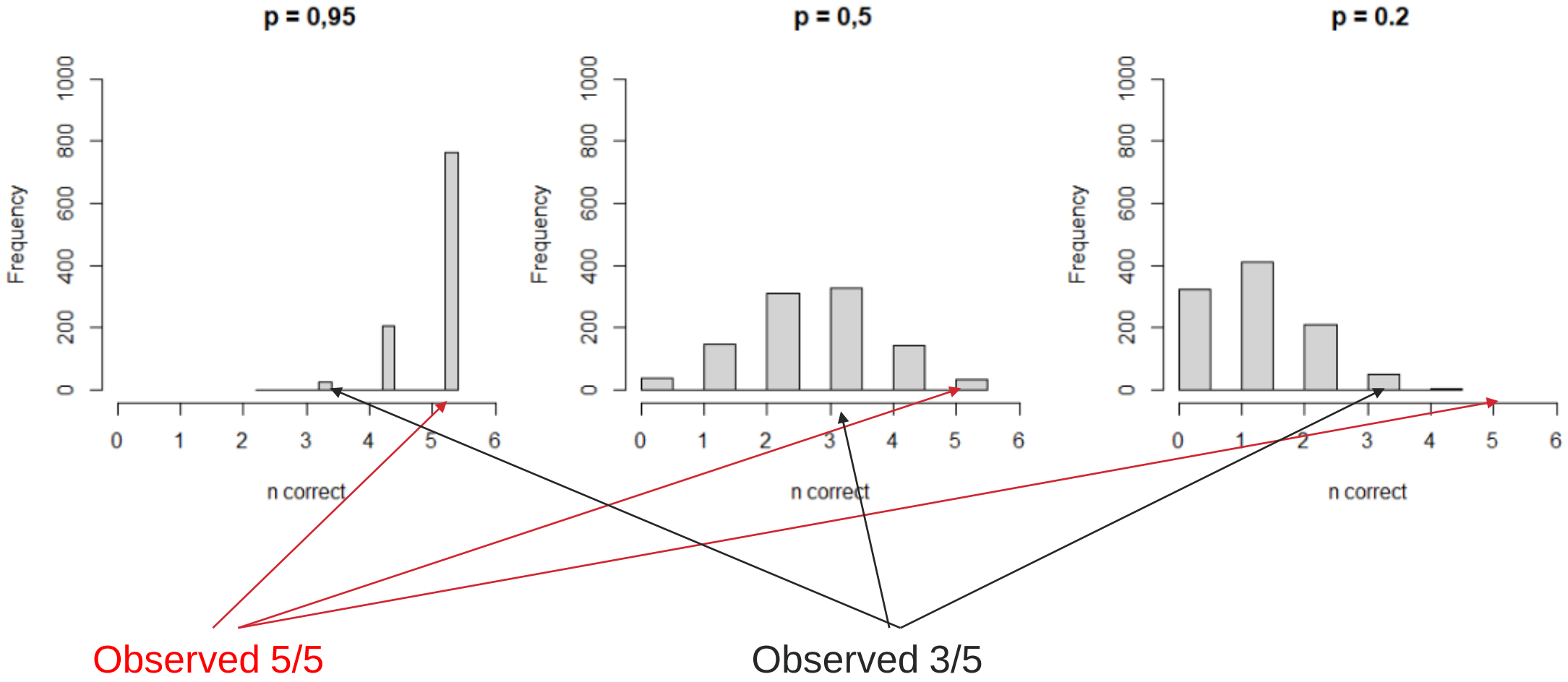


If 90% of colonies are correct -> p= 0.9

If 30 % of colonies are correct -> p= 0.3



# CAN I ESTIMATE P FROM 5 COLONIES?

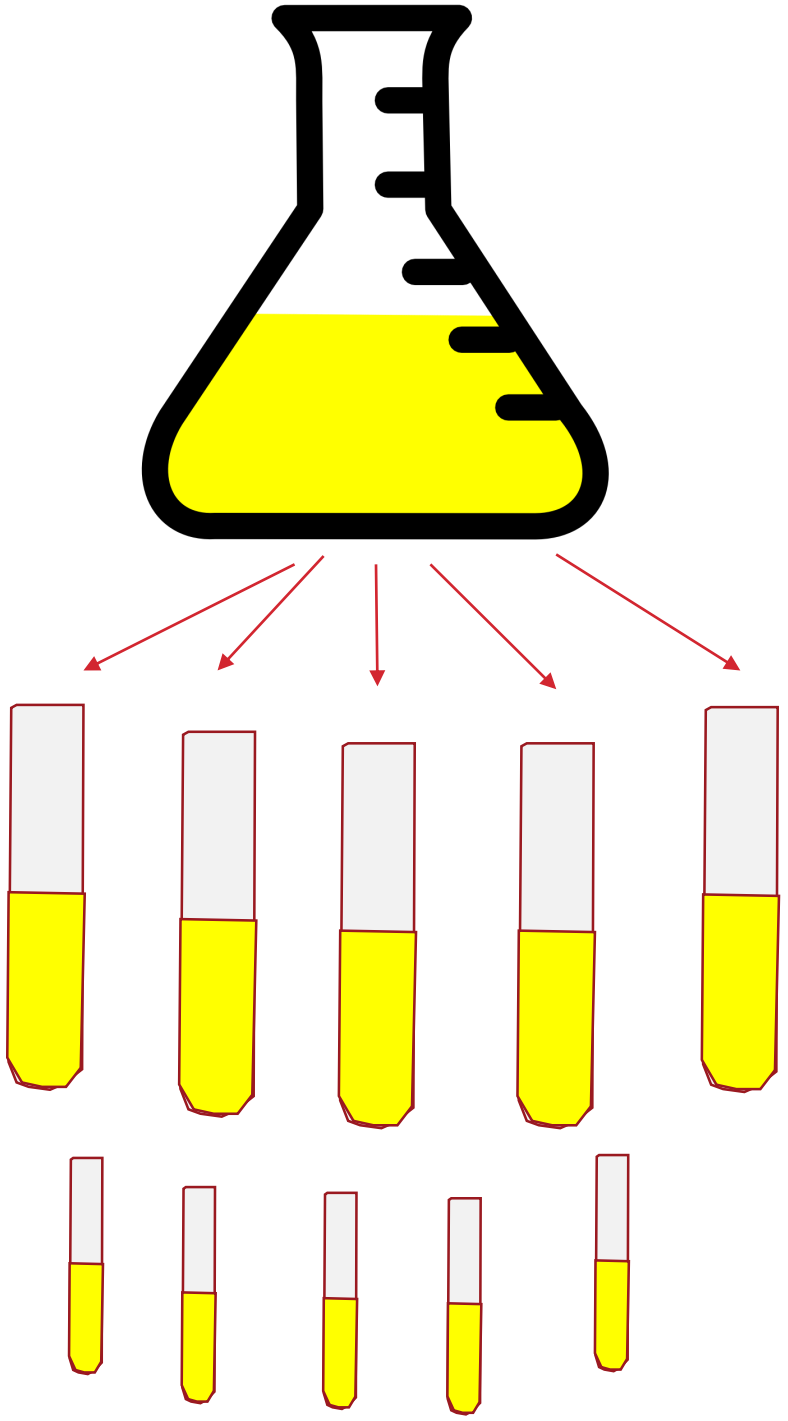


# SUMMARY CONFIRMATION UNCERTAINTY

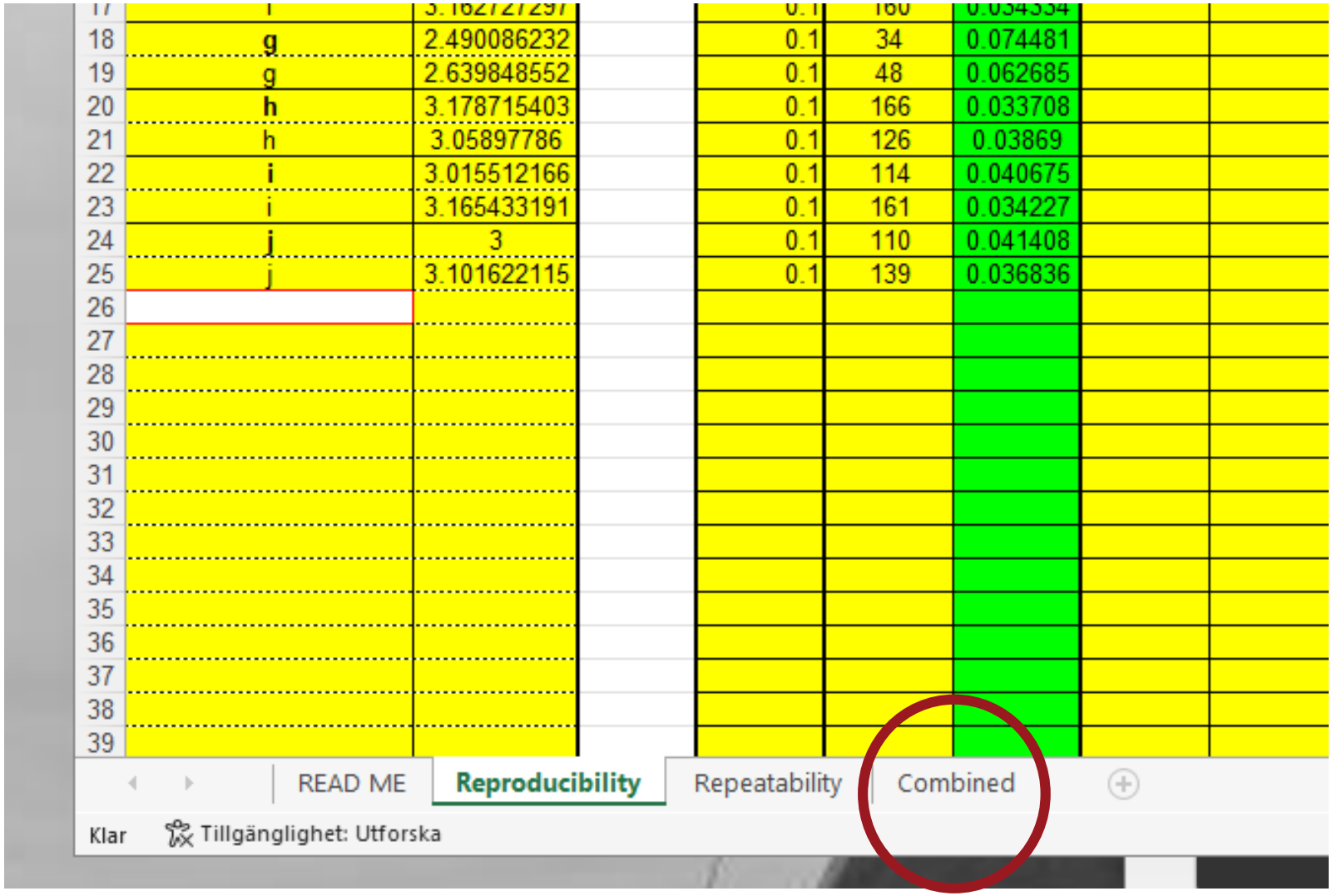
- When calculating the CFU/ml I will correct the value using my estimate of p
- If 4/5 samples are positive, my estimate of p is 0.8
- Other values of p cannot be excluded, thus *uncertainty*
- Fewer picked colones and fewer positives means larger uncertainty

# MPN UNCERTAINTY

- Concentration is estimated from serial dilutions
- Number of colonies in each tube is poisson distributed
- Growth if more than one
- Uncertainty of mpn estimate depends on both the setup and the results
- Implemented in ISO19036 tool



# ISO-19036 CALCULATION TOOL



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17 | f | 3.162727297 | | 0.1 | 160 | 0.034334 | | |
| 18 | **g** | 2.490086232 | | 0.1 | 34 | 0.074481 | | |
| 19 | g | 2.639848552 | | 0.1 | 48 | 0.062685 | | |
| 20 | **h** | 3.178715403 | | 0.1 | 166 | 0.033708 | | |
| 21 | h | 3.05897786 | | 0.1 | 126 | 0.03869 | | |
| 22 | **i** | 3.015512166 | | 0.1 | 114 | 0.040675 | | |
| 23 | i | 3.165433191 | | 0.1 | 161 | 0.034227 | | |
| 24 | **j** | 3 | | 0.1 | 110 | 0.041408 | | |
| 25 | j | 3.101622115 | | 0.1 | 139 | 0.036836 | | |
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READ ME | **Reproducibility** | Repeatability | Combined

Klar | Tillgänglighet: Utforska

# CALCULATIONS IN EXCEL TOOL

Calculate MU accounting for Poisson uncertainty for a laboratory sample in order to report results.

Prerequisite: You have previously obtained estimates for matrix uncertainty and technical uncertainty

# EXCEL CALCULATIONS TOOL

- 4rd worksheet “Combines”
  - Estimate MU
    = to estimate the matrix uncertainty

# DATA

| | | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | | | Contact person | | Email address | |
| Country | | | GDPR agreement | Yes | | |
| **Method** | | | **Repeatability conditions for test portions of the same sample** | | **Matrix tested** | |
| Target organism | Total mesophilic aerobic count | | Operator repeatability | Yes | Category | Pet food and animal feed |
| Method | ISO 4833-1:2013 | | Equipment repeatability | Yes | Type | Animal feeds (fish) |
| Accreditation | Yes | | Media and reagents repeatability | Yes | Matrix | Flour |
| General comment on the method or matrix | | | Weight of each test portion 10 g | Yes | Additional description | |

| | Sample and conditions | | | | Results | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ref_n | sample_ID | test_portion_ID | start_date | start_time | dilution_1 | volume_1 | colony_count_dil1_plate1 | colony_count_dil1_plate2 | dilution_2 | volume_2 | colony | colony | confirme |
| 1 | 1 | 1a | 2021-10-08 | 09:30 | -2 | 1.0 | 109 | | -3 | 1.0 | 14 | | |
| 2 | 1 | 1b | 2021-10-08 | 09:30 | -2 | 1.0 | 87 | | -3 | 1.0 | 8 | | |
| 3 | 1 | 1c | 2021-10-08 | 09:30 | -2 | 1.0 | 143 | | -3 | 1.0 | 19 | | |
| 4 | 1 | 1d | 2021-10-08 | 09:30 | -2 | 1.0 | 99 | | -3 | 1.0 | 12 | | |
| 5 | 1 | 1e | 2021-10-08 | 09:30 | -2 | 1.0 | 130 | | -3 | 1.0 | 12 | | |
| 6 | 1 | 1f | 2021-10-08 | 09:30 | -2 | 1.0 | 86 | | -3 | 1.0 | 8 | | |
| 7 | 1 | 1g | 2021-10-08 | 09:30 | -2 | 1.0 | 99 | | -3 | 1.0 | 8 | | |
| 8 | 1 | 1h | 2021-10-08 | 09:30 | -2 | 1.0 | 102 | | -3 | 1.0 | 9 | | |
| 9 | 1 | 1i | 2021-10-08 | 09:30 | -2 | 1.0 | 111 | | -3 | 1.0 | 10 | | |
| 10 | 1 | 1j | 2021-10-08 | 09:30 | -2 | 1.0 | 138 | | -3 | 1.0 | 18 | | |
| 11 | 1 | 1k | 2021-10-08 | 09:30 | -2 | 1.0 | 112 | | -3 | 1.0 | 14 | | |
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| 20 | | | | | | | | | | | | | |

- Make new columns with appropriate formulae
- Calculate conc and log10conc - as before
- Calculate the number of colonies used – for each sample

| | | | | |
|---|---|---|---|---|
| | **Operator repeatability** | Yes | **Category** | Raw meat and ready-to-cook meat products (except poultry) |
| | **Equipment repeatability** | Yes | **Type** | Fresh meats (unprocessed) |
| | **Media and reagents repeatability** | Yes | **Matrix** | Pork meat cuts |
| | **Weight of each test portion 10 g** | Yes | **Additional description** | Laboratory samples - portions cut of pork meat (300-500g), refrigerated, collected from retail |

**Results**

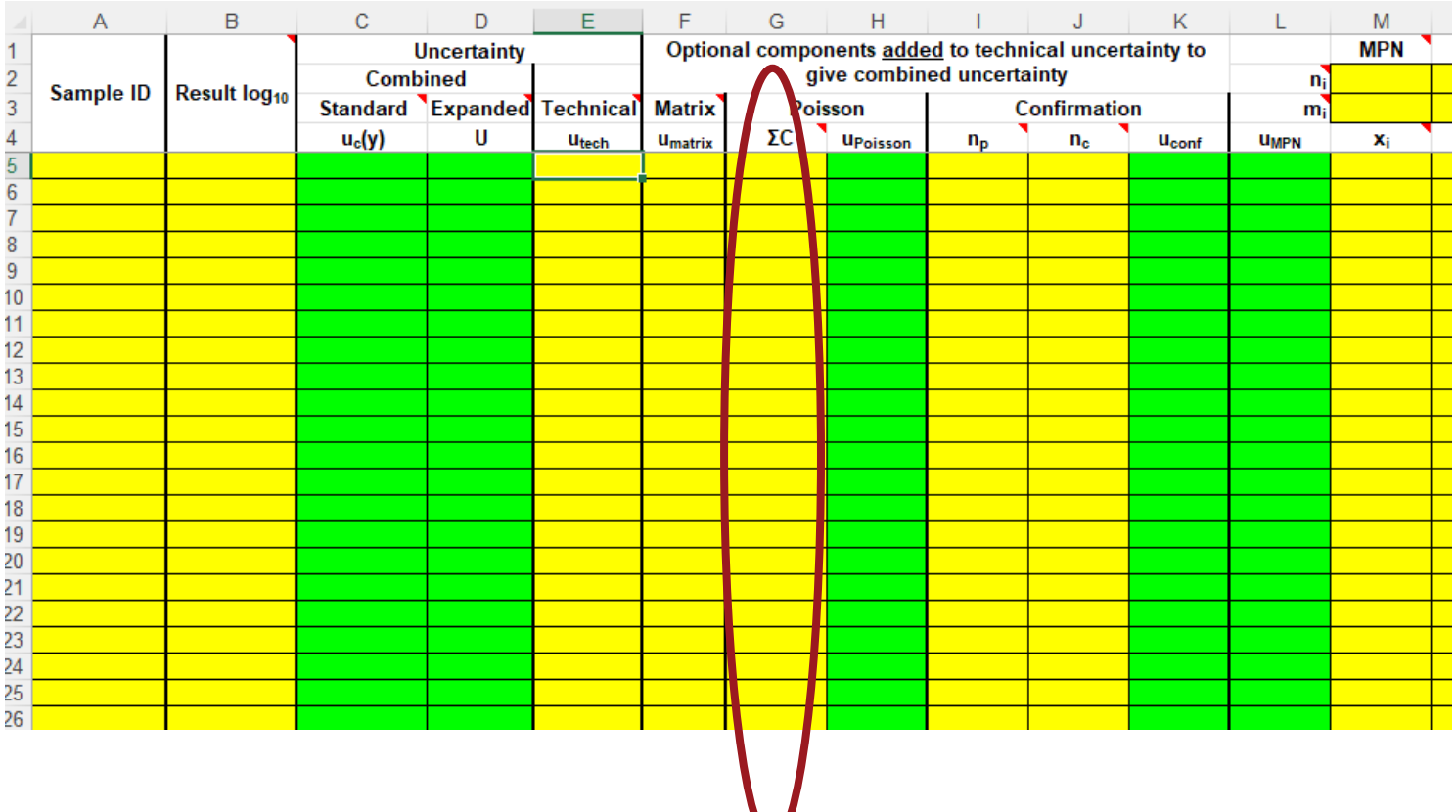
| *dilution_1* | *volume_1* | *colony_count_dil1_plate1* | *colony_count_dil1_plate2* | *dilution_2* | *volume_2* | *colony_* | *colony_* | *confirmed* | *comment* |
|---|---|---|---|---|---|---|---|---|---|
| −1 | 1.0 | 76 | | −2 | 1.0 | 6 | | | |
| −1 | 1.0 | 42 | | −2 | 1.0 | 6 | | | |
| −1 | 1.0 | 114 | | −2 | 1.0 | 9 | | | |
| −1 | 1.0 | 66 | | −2 | 1.0 | 6 | | | |
| −2 | 1.0 | 180 | | −3 | 1.0 | 15 | | | |
| −2 | 1.0 | 259 | | −3 | 1.0 | 23 | | | |
| −1 | | 156 | | −2 | 1.0 | 14 | | | |
| −1 | | 113 | | −2 | 1.0 | 10 | | | |
| −3 | | 65 | | −4 | 1.0 | 9 | | | |
| −3 | | 41 | | −4 | 1.0 | 6 | | | |
| −3 | | 99 | | −4 | 1.0 | 8 | | | |
| −3 | | 143 | | −4 | 1.0 | 17 | | | |
| −3 | 1.0 | 30 | | −4 | 1.0 | 4 | | | |
| −3 | 1.0 | 43 | | −4 | 1.0 | 5 | | | |
| −2 | 1.0 | 154 | | −3 | 1.0 | 12 | | | |
| −2 | 1.0 | 118 | | −3 | 1.0 | 8 | | | |
| −2 | 1.0 | 103 | | −3 | 1.0 | 11 | | | |
| −2 | 1.0 | 142 | | −3 | 1.0 | 19 | | | |
| −3 | 1.0 | 99 | | −4 | 1.0 | 11 | | | |
| −3 | 1.0 | 127 | | −4 | 1.0 | 12 | | | |

Indicate the volume inoculated (in ml) of the first dilution to use in calculation, e.g. 0.1, 1.0 (or 1,0, 0,1 etc, depending on format in Excel).

How would you make the formula?

# ENTER DATA IN TOOL

- Enter data – check README for format *etc.*
- Make sure that there is no formatting!
  (numbers are numbers, not text)
- Use technical and ,matrix uncertainty from before.



| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sample ID | Result $\log_{10}$ | Uncertainty | | | Optional components added to technical uncertainty to give combined uncertainty | | | | | | | MPN |
| 2 | | | Combined | | | | | | | | | $n_i$ | |
| 3 | | | Standard | Expanded | Technical | Matrix | Poisson | | Confirmation | | | $m_i$ | |
| 4 | | | $u_c(y)$ | U | $u_{tech}$ | $u_{matrix}$ | $\Sigma C$ | $u_{Poisson}$ | $n_p$ | $n_c$ | $u_{conf}$ | $u_{MPN}$ | $x_i$ |
| 5 | | | | | | | | | | | | | |
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| 26 | | | | | | | | | | | | | |

# QUESTIONS

- How much larger MU will you get when considering Poisson-uncertainty compared to only using technical and matrix uncertainty.

- How big is the difference in MU between the samples with the highest count compared with the lowest.

# WHEN IS POISSON DISTRIBUTION A PROBLEM

| Sample ID | Result $\log_{10}$ | Uncertainty | | | Optional components added to technical uncertainty to give combined uncertainty | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Combined | | | | Poisson | | Confirmation | | | |
| | | Standard | Expanded | Technical | Matrix | | | | | | |
| | | $u_c(y)$ | U | $u_{tech}$ | $u_{matrix}$ | ΣC | $u_{Poisson}$ | $n_p$ | $n_c$ | $u_{conf}$ | $u_I$ |
| A | 2.405765346 | 0.190743263 | 0.3814865 | 0.15 | 0.10953 | 100 | 0.0434294 | | | | |
| B | 2.320335151 | 0.19562492 | 0.3912498 | 0.15 | 0.10953 | 50 | 0.0614185 | | | | |
| C | 2.28082661 | 0.20195032 | 0.4039006 | 0.15 | 0.10953 | 30 | 0.079291 | | | | |
| D | 2.356547324 | 0.230993604 | 0.4619872 | 0.15 | 0.10953 | 10 | 0.137336 | | | | |
| E | 2.356547324 | 0.268736329 | 0.5374727 | 0.15 | 0.10953 | 5 | 0.1942224 | | | | |
| | | | | | | | | | | | |
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# ADDING BY THE SQUARES

| | | | | U tot |
|---|---|---|---|---|
| U1 | U2 | U1^2 | U1^2 | root(U1^2 + U1^2) |

# CONFIRMATION UNCERTAINTY IN MU ESTIMATION

| | Sample and conditions | | | | Results | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ref_no | sample_ID | test_portion_ID | start_date | start_time | dilution_1 | volume_1 | colony_count_dil1_plate1 | colony_count_dil1_plate2 | dilution_2 | volume_2 | colony_ | colony_ | confirmed |
| 1 | 1 | 902 | A | 2021-07-21 | 10:00 | 0 | 1.0 | 25 | | −1 | 1.0 | 3 | | 5/5 |
| 2 | 2 | 902 | B | 2021-07-21 | 10:00 | 0 | 1.0 | 21 | | −1 | 1.0 | 2 | | 5/5 |
| 3 | 3 | 902 | C | 2021-07-21 | 10:00 | 0 | 1.0 | 19 | | −1 | 1.0 | 2 | | 3/3 |
| 4 | 4 | 902 | D | 2021-07-21 | 10:00 | 0 | 1.0 | 23 | | −1 | 1.0 | 2 | | 3/3 |
| 5 | 5 | 902 | E | 2021-07-21 | 10:00 | 0 | 1.0 | 24 | | −1 | 1.0 | 2 | | 4/4 |
| 6 | 6 | 902 | F | 2021-07-21 | 10:00 | 0 | 1.0 | 25 | | −1 | 1.0 | 3 | | 4/4 |
| 7 | 7 | 902 | G | 2021-07-21 | 10:00 | 0 | 1.0 | 27 | | −1 | 1.0 | 3 | | 3/3 |
| 8 | 8 | 902 | H | 2021-07-21 | 10:00 | 0 | 1.0 | 30 | | −1 | 1.0 | 3 | | 5/5 |
| 9 | 9 | 902 | I | 2021-07-21 | 10:00 | 0 | 1.0 | 26 | | | 1.0 | 3 | | 5/5 |
| 10 | 10 | 902 | J | 2021-07-21 | 10:00 | 0 | 1.0 | 26 | | | 1.0 | 3 | | 2/2 |
| 11 | 11 | 902 | K | 2021-07-21 | 10:00 | 0 | 1.0 | 27 | | | 1.0 | 3 | | 3/3 |
| 12 | 12 | | | | | | | | | | | | | |
| 13 | 13 | | | | | | | | | | | | | |

Indicate the number of typical* colonies counted on the second plate of the first dilution.

* Presumptive colonies if the method includes a confirmation

# HINT

- Possible to use special functions in excel

| confirmed_colonies | *konc* | *log10conc* | *räknade* | $n_p$ | $n_c$ | corrected |
|---|---|---|---|---|---|---|
| 5/5 | 254.5455 | 2.405765 | 28 | 5 | 5 | 254.5455 |
| 5/5 | 209.0909 | 2.320335 | 23 | 5 | 5 | 209.0909 |

=TALVÄRDE(TEXTEFTER(N11;"/"))

”make numeric the text after delimitor ”/”

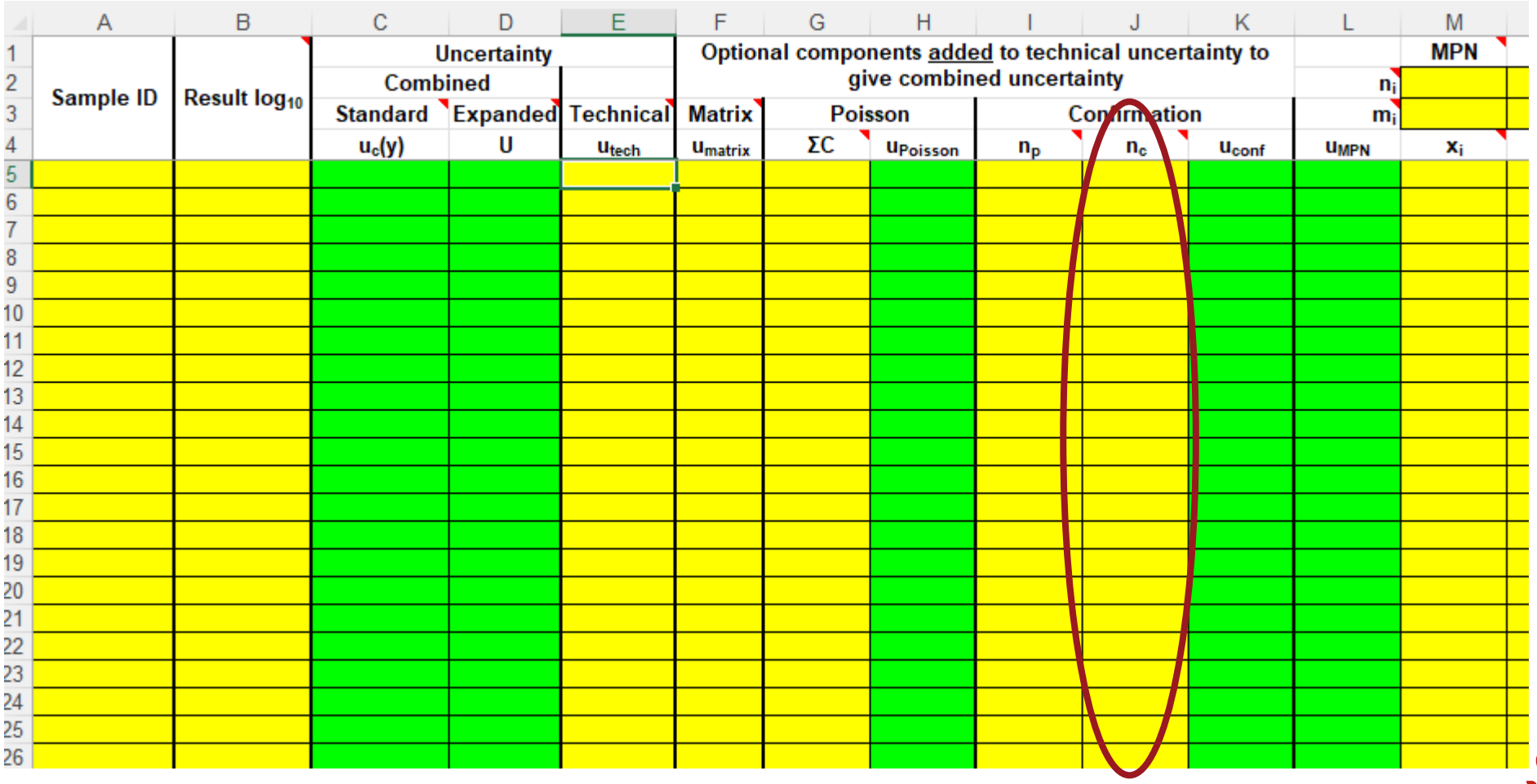
| confirmed_colonies | *konc* | *log10conc* | *räknade* | $n_p$ | $n_c$ | corrected |
|---|---|---|---|---|---|---|
| 5/5 | 254.5455 | 2.405765 | 28 | 5 | 5 | 254.5455 |
| 5/5 | 209.0909 | 2.320335 | 23 | 5 | 5 | 209.0909 |

=P11*S11/T11

# ENTER DATA IN TOOL

Enter data – check README for format *etc.*

*Use technical and ,matrix uncertainty from before.*



| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sample ID | Result $\log_{10}$ | Uncertainty | | | Optional components added to technical uncertainty to give combined uncertainty | | | | | | | MPN |
| 2 | | | Combined | | | | | | | | | $n_j$ | |
| 3 | | | Standard | Expanded | Technical | Matrix | Poisson | | Confirmation | | | $m_j$ | |
| 4 | | | $u_c(y)$ | U | $u_{tech}$ | $u_{matrix}$ | ΣC | $u_{Poisson}$ | $n_p$ | $n_c$ | $u_{conf}$ | $u_{MPN}$ | $x_j$ |
| 5 | | | | | | | | | | | | | |
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| 26 | | | | | | | | | | | | | |

# QUESTIONS

- How much larger MU will you get when considering confirmation-uncertainty compared to only using technical and matrix uncertainty.

- How much larger MU will you get when considering both poisson- and confirmation-uncertainty compared to only using technical and matrix uncertainty.

- How big is the difference in MU between the samples with the highest confirmation uncertainty compared with those with the lowest.

# WHEN IS CONFIRMATION A PROBLEM?

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sample ID | Result $\log_{10}$ | Uncertainty | | | Optional components added to technical uncertainty to give combined uncertainty | | | | | | |
| 2 | | | Combined | | | | | | | | | $n_i$ |
| 3 | | | Standard | Expanded | Technical | Matrix | Poisson | | Confirmation | | | $m_i$ |
| 4 | | | $u_c(y)$ | U | $u_{tech}$ | $u_{matrix}$ | ΣC | $u_{Poisson}$ | $n_p$ | $n_c$ | $u_{conf}$ | $u_{MPN}$ |
| 5 | A | 2.405765346 | 0.208065542 | 0.4161311 | 0.15 | 0.10953 | 28 | 0.0820739 | 5 | 5 | 0.0453681 | |
| 6 | B | 2.320335151 | 0.213393175 | 0.4267864 | 0.15 | 0.10953 | 21 | 0.0947708 | 5 | 5 | 0.0453681 | |
| 7 | C | 2.28082661 | 0.235406686 | 0.4708134 | 0.15 | 0.10953 | 10 | 0.137336 | 5 | 5 | 0.0453681 | |
| 8 | D | 2.356547324 | 0.272538946 | 0.5450779 | 0.15 | 0.10953 | 5 | 0.1942224 | 5 | 5 | 0.0453681 | |
| 9 | | | | | | | | | | | | |
| 10 | E | 2.405765346 | 0.20990994 | 0.4198199 | 0.15 | 0.10953 | 28 | 0.0820739 | 4 | 4 | 0.05319 | |
| 11 | F | 2.43572857 | 0.212976263 | 0.4259525 | 0.15 | 0.10953 | 28 | 0.0820739 | 3 | 3 | 0.064233 | |
| 12 | G | 2.477121255 | 0.269570566 | 0.5391411 | 0.15 | 0.10953 | 28 | 0.0820739 | 4 | 2 | 0.1773 | |
| 13 | H | 2.421005313 | 0.383217377 | 0.7664348 | 0.15 | 0.10953 | 28 | 0.0820739 | 4 | 1 | 0.3249962 | |
| 14 | | | | | | | | | | | | |
| 15 | K | 2.43572857 | 0.270648637 | 0.5412973 | 0.15 | 0.10953 | 10 | 0.137336 | 3 | 2 | 0.1410413 | |
| 16 | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | |

# GOOD LUCK