# Exploring the rationale for the high concentration of aluminium used in clinical vaccinations.


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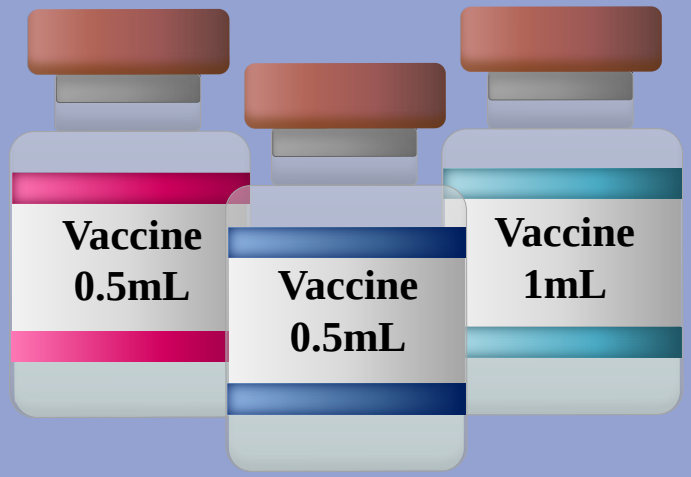
# Introduction




**How does the concentration of Al in vaccines impact the biology at the site of injection?**

**Maximum amount of Al permitted in vaccines [1]**

- 0.85mg/dose if determined by assay
- 1.14mg/dose if determined by calculation on basis of Al compound added

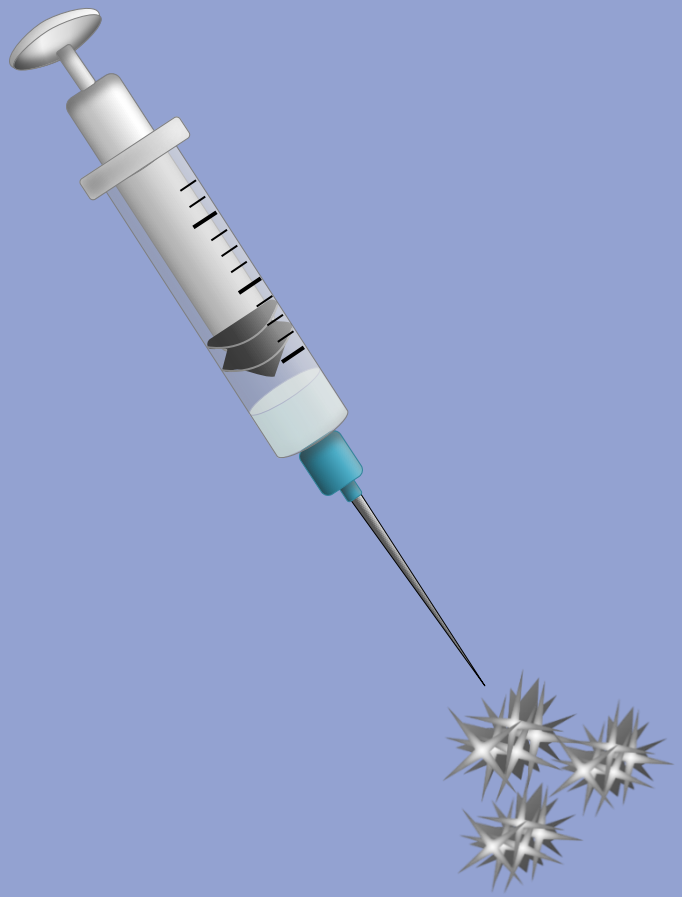
**Average amount of Al in vaccines**

- *ca* 0.4mg/dose (0.8mg/mL)

**Rationale**

- Higher concentrations of Al are more effective.
  - >0.5mg/dose Al did not improve efficacy of tetanus vaccine [2].
  - High concentrations of Al may impede immunological response – cytotoxicity [3]

# Methodological approach



## Vaccine models

- Alhydrogel in saline (no antigen) – 0.3-0.9mg/mL Al (pH 7)

## Physicochemical characterisation

- Particle size
- Zeta potential
- Size exclusion filtration/GFAAS (Al quantification)

## Uptake

- Fluorescence microscopy - lumogallion staining (50µM)
- Macrophages exposed to Al for 1hr

## Cell viability

- Presto blue assay – determinant of metabolic activity (resazurin based assay)

# DLS – Particle size distribution vs. concentration of Al in simulated vaccines.

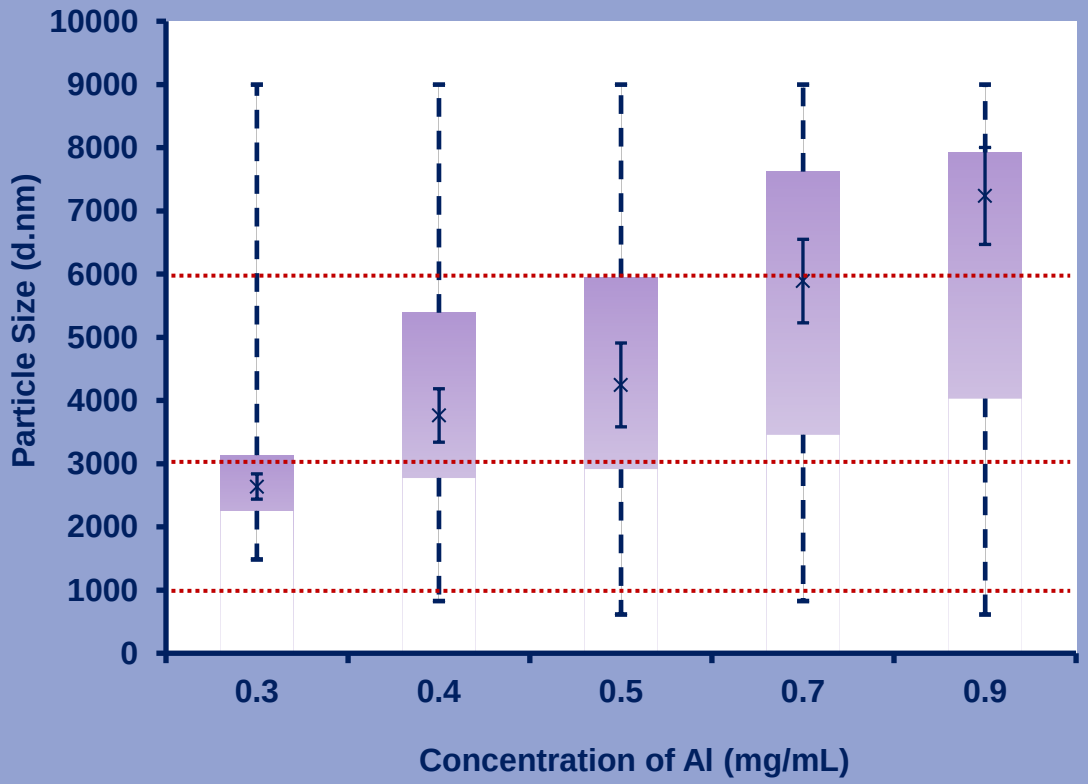



***Fig 1: Particle size distributions of Alhydrogel only vaccines containing 0.3-0.9mg/mL Al. Purple boxes indicate the interquartile range of the data while the dashed bars show the span. Blue crosses show the average d50 values and d50 error bars represent the ±SD of the measurement where n=5. The red dotted lines highlight the relevant filtration size cut-offs used in complementary experiments.***

- Interquartile range encompassed larger particles as the concentration of Al was increased.
- The breadth of the interquartile range also increased when the concentration of Al was increased.
- Based on the theoretical filtration size cut-offs:
  - ❖ The majority of the Al in these vaccines will exist as micron-sized aggregates i.e. >1µm.
  - ❖ Significant shifts in size are expected between 0.3 & 0.4mg/mL and 0.5 & 0.7mg/mL.

# DLS – PDI vs. concentration of Al in simulated vaccines.

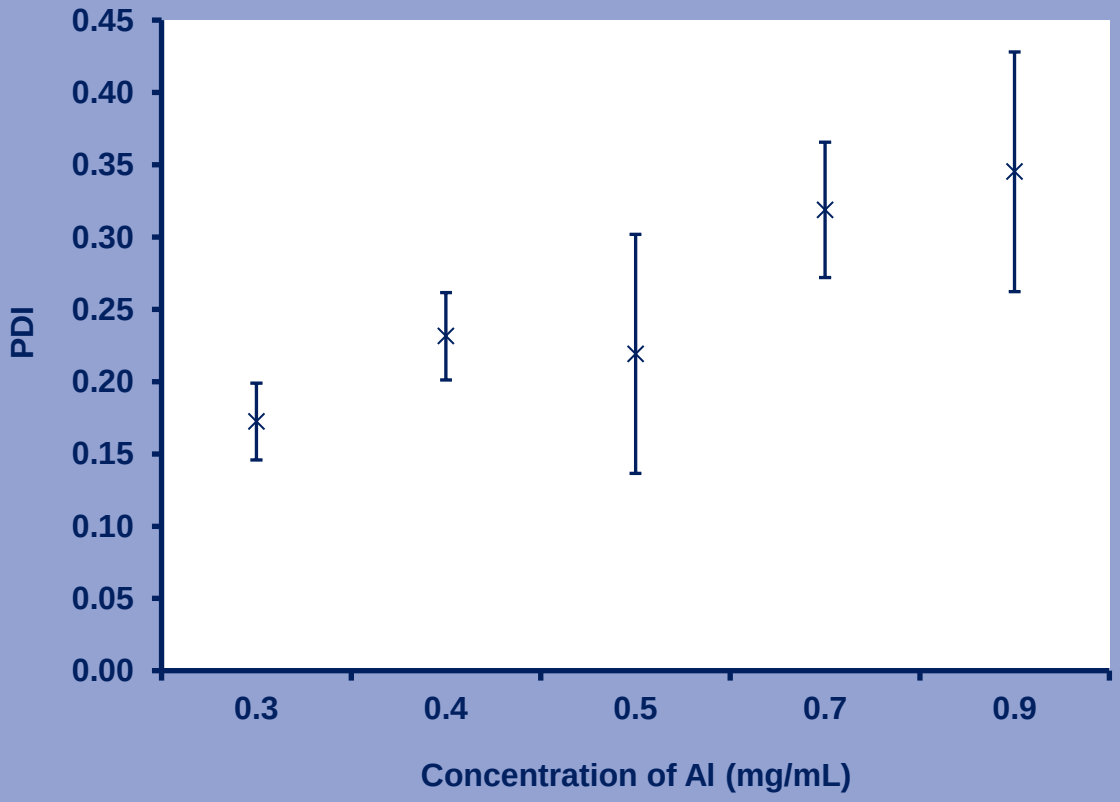



***Fig 2: PDI of Alhydrogel only vaccines containing 0.3-0.9mg/mL Al. Error bars represent the ±SD of the measurement where n=5***

- PDI values predominantly increased over the concentration range studied (0.172-0.345).
- Significant difference in PDI observed between :
  - 0.3 & 0.9mg/mL (0.172 vs. 0.345, P=0.001).
  - 0.4 & 0.7 mg/mL Al (0.231 vs. 0.319, P=0.05).

# DLS – Median particle size vs. concentration of Al in simulated vaccines.

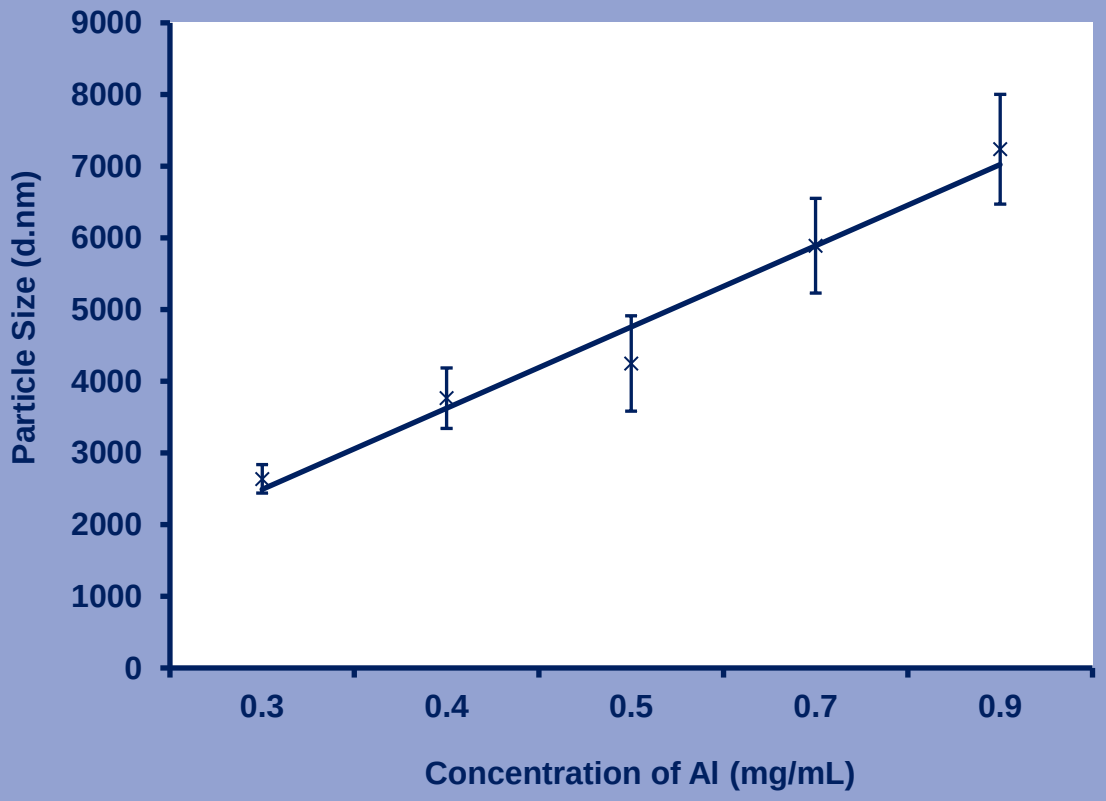



***Fig 3: Particle size (d50) of Alhydrogel only vaccines containing 0.3-0.9mg/mL Al. Error bars represent the ±SD of the measurement where n=5***

- D50 values increased in a linear manner over the concentration range studied (2638 -7237 nm , $R^2 = 0.974$).
- Significant difference in median particle size between:
  - 0.3 & 0.9 mg/mL (2638 nm vs. 7237 nm, P<0.0001).
  - 0.3 & 0.4 mg/mL (2638 nm vs. 3764 nm, P=0.03)
  - 0.5 & 0.7 mg/mL (4247 nm vs. 5890 nm, P=0.0007).

# ELS – Zeta potential vs. concentration of Al in simulated vaccines.

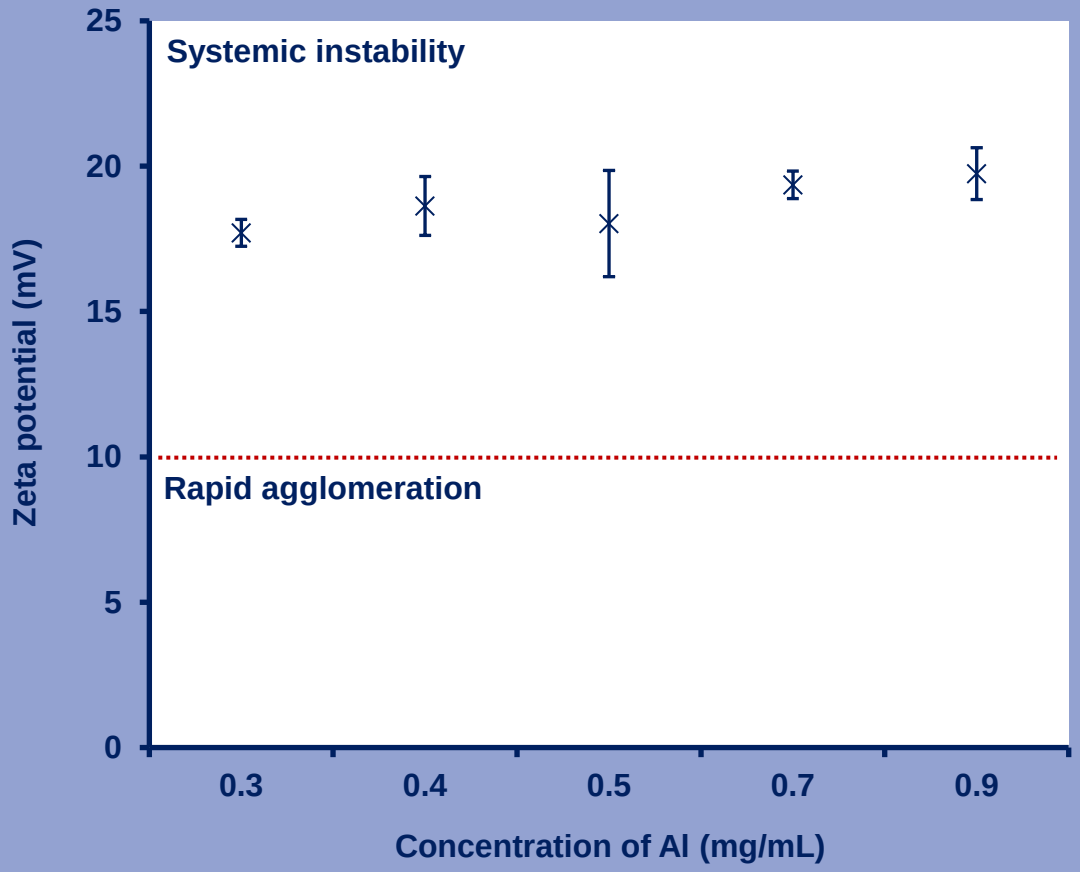
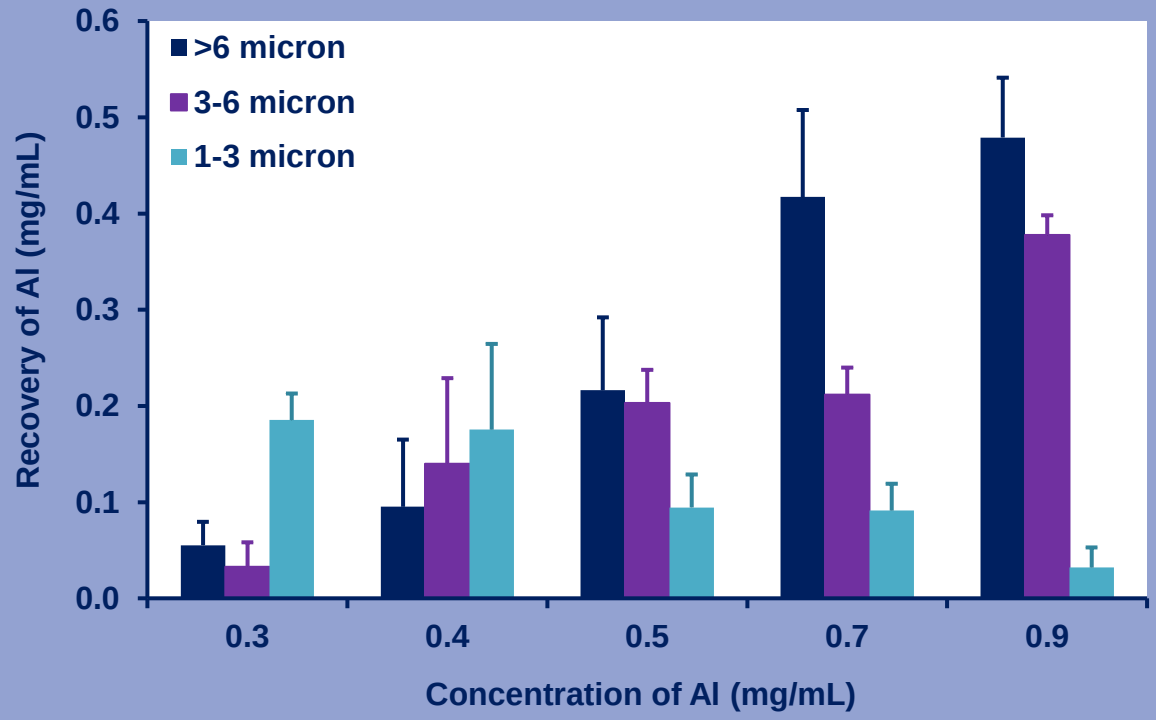



*Fig 4: Zeta potential of Alhydrogel only vaccines containing 0.3-0.9mg/mL Al. Error bars represent the ±SD of the measurement where n=5*

- Zeta potential values remained fairly consistent over the concentration range studied and were located in the region associated with systemic instability (17.71-19.74mV).
- Lowest vaccine dose had a significantly lower zeta potential than that of the highest vaccine dose (17.71 vs. 19.74 mV, P=0.05).
- Vaccine particles are positively charged at pH 7

# GFAAS/filtration – % aluminium recovery vs. concentration of Al in simulated vaccines.




- Abundance of particles between 1-3µm decreased when concentration of Al increased.
- Significant differences observed between:
  - ❖ 0.3mg/mL & 0.9mg/mL (0.19mg/mL vs. 0.03mg/mL, P=0.004)
  - ❖ 0.4mg/mL & 0.9mg/mL (0.18mg/mL vs. 0.03mg/mL, P=0.01)
- Smaller particles present at 0.3mg/mL vs. 0.9mg/mL – lower concentrations are more likely to be internalised by macrophages

| Concentration of Al (mg/mL) | 0.3 | 0.4 | 0.5 | 0.7 | 0.9 |
|---|---|---|---|---|---|
| | Recovery of Al (%) | | | | |
| 1-3µm | 77.06 | 55.04 | 10.89 | 3.45 | 0.68 |
| 3-6µm | 2.71 | 17.30 | 47.02 | 33.64 | 30.09 |
| >6µm | 20.19 | 27.62 | 42.08 | 62.90 | 69.20 |

***Fig 5: The relative abundance of aluminium (%) within specific particle size fractions for Alhydrogel only vaccines containing an initial concentration of 0.3-0.9mg/mL Al. Blue, purple and green boxes represent the % Al in the size fractions >6µm, 3-6µm & 1-3µm respectively.***

# DLS – Particle size distribution of simulated vaccines in R10 medium (1hr incubation)

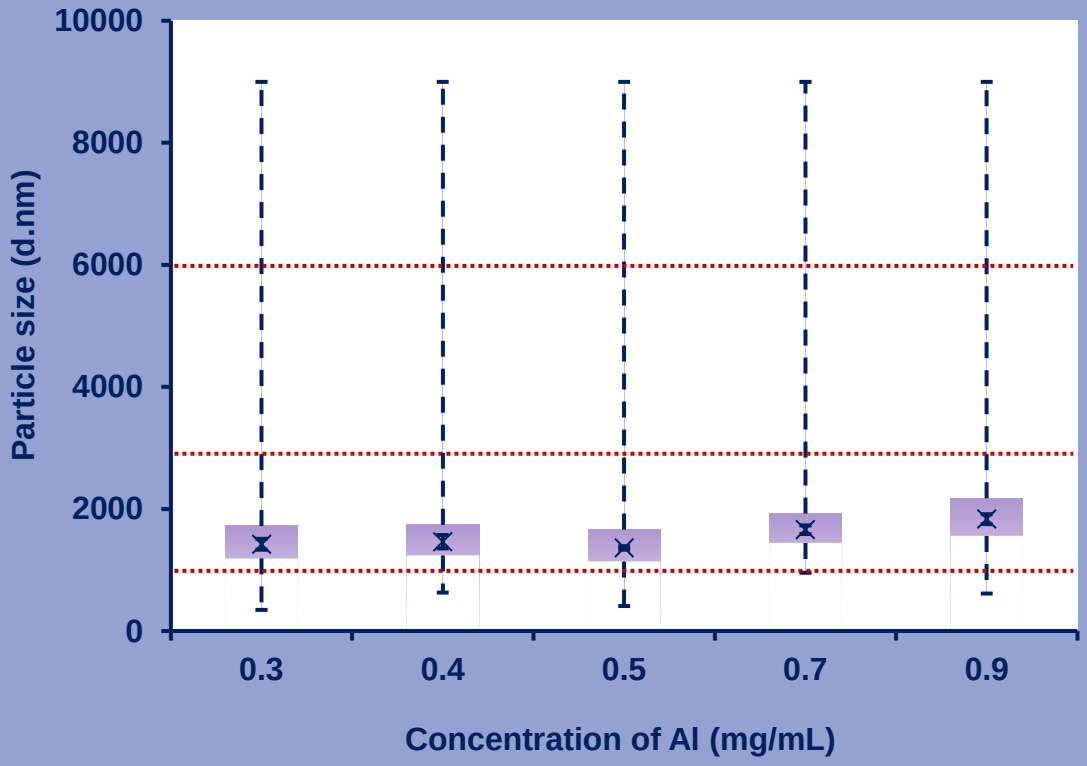



***Fig 6: Particle size distributions of Alhydrogel only vaccines 0.3-0.9mg/mL Al following 1hr incubation within R10 medium (37°C). Purple boxes indicate the interquartile range of the data while the dashed bars show the span. Blue crosses show the average d50 values and d50 error bars represent the ±SD of the measurement where n=5. The red dotted lines highlight the relevant filtration size cut-offs used in complementary experiments.***

- Interquartile range of the data remained consistent as the concentration of Al was increased.
- The breadth of the interquartile range also remained stable as the concentration of Al was increased.
- Based on the theoretical filtration size cut-offs:
  - ❖ The majority of the Al will exist as micron-sized aggregates i.e. >1µm.
  - ❖ Significant shifts in size between concentrations are unlikely.

# DLS – PDI of simulated vaccines in R10 medium (1hr incubation)

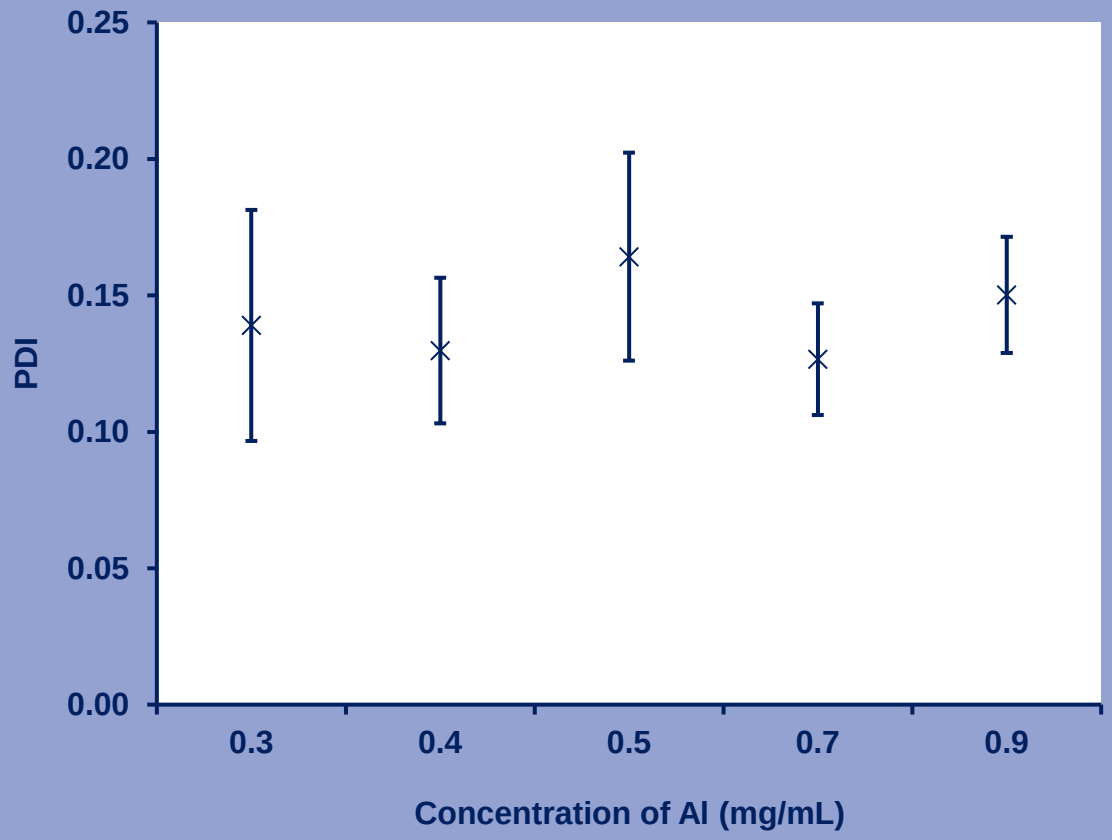



*Fig 7: PDI of Alhydrogel only vaccines containing 0.3-0.9mg/mL Al following 1hr incubation within R10 medium (37°C). Error bars represent the ±SD of the measurement where n=5*

- PDI values remained stable over the concentration range studied (0.127-0.164).
- No significant difference in PDI between any of the concentrations studied.

# DLS – Median particle size vs. concentration of Al in simulated vaccines.

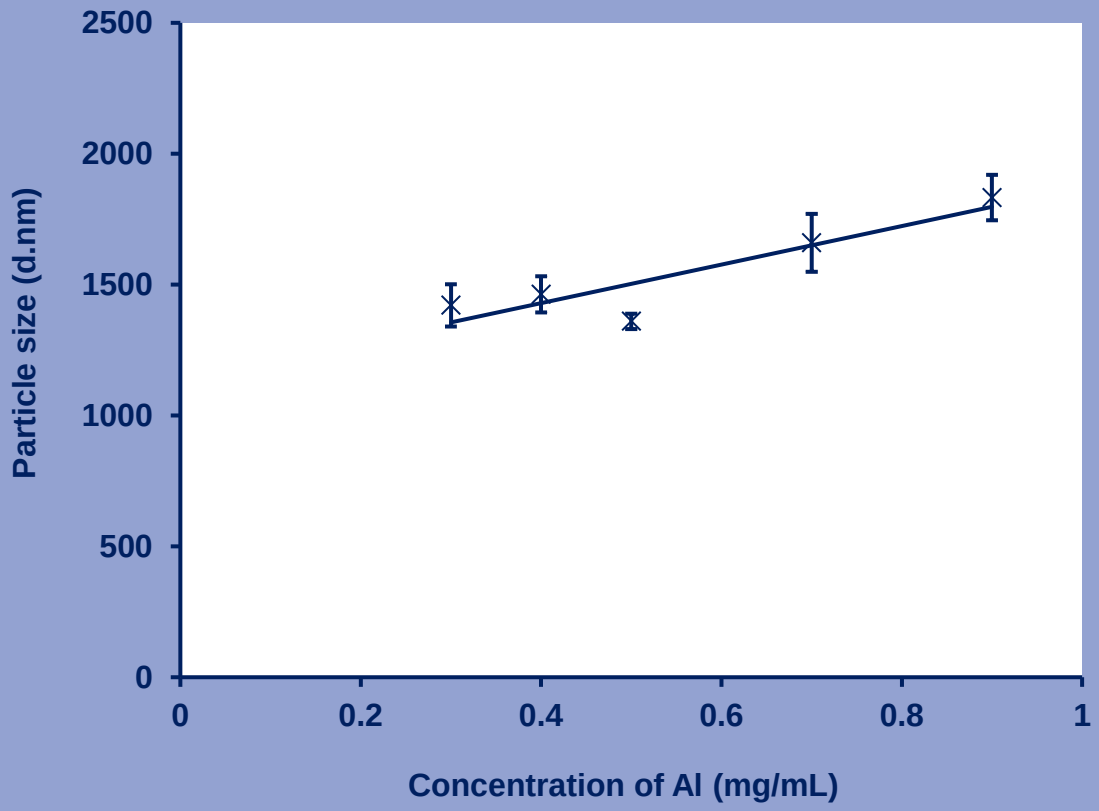
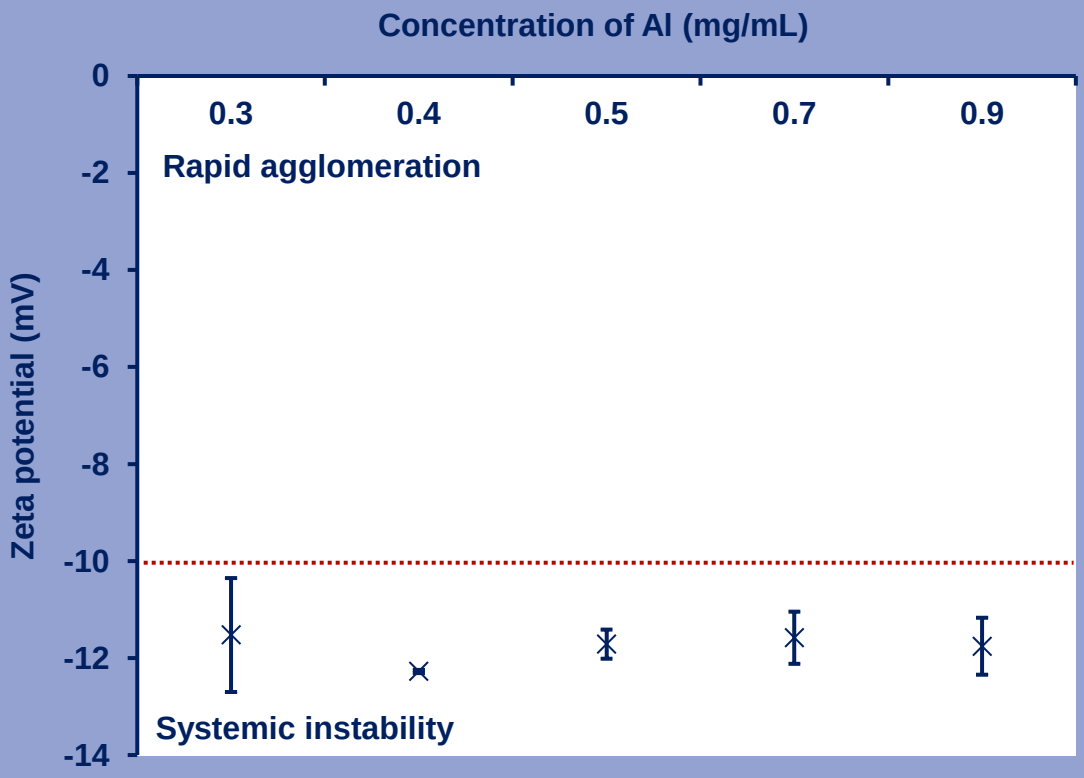



***Fig 8: Particle size (d50) of Alhydrogel only vaccines containing 0.3-0.9mg/mL Al following 1hr incubation within R10 medium (37°C). Error bars represent the ±SD of the measurement where n=5***

- D50 values increased in a weakly linear manner over the concentration range studied (2638 -7237nm , $R^2 = 0.822$).
- Significant difference in particle size between:
  - 0.3 & 0.9 mg/mL (1421 nm vs. 1833 nm, P<0.0001).
  - 0.5 & 0.7 mg/mL (1360 nm vs. 1660 nm, P<0.0001).

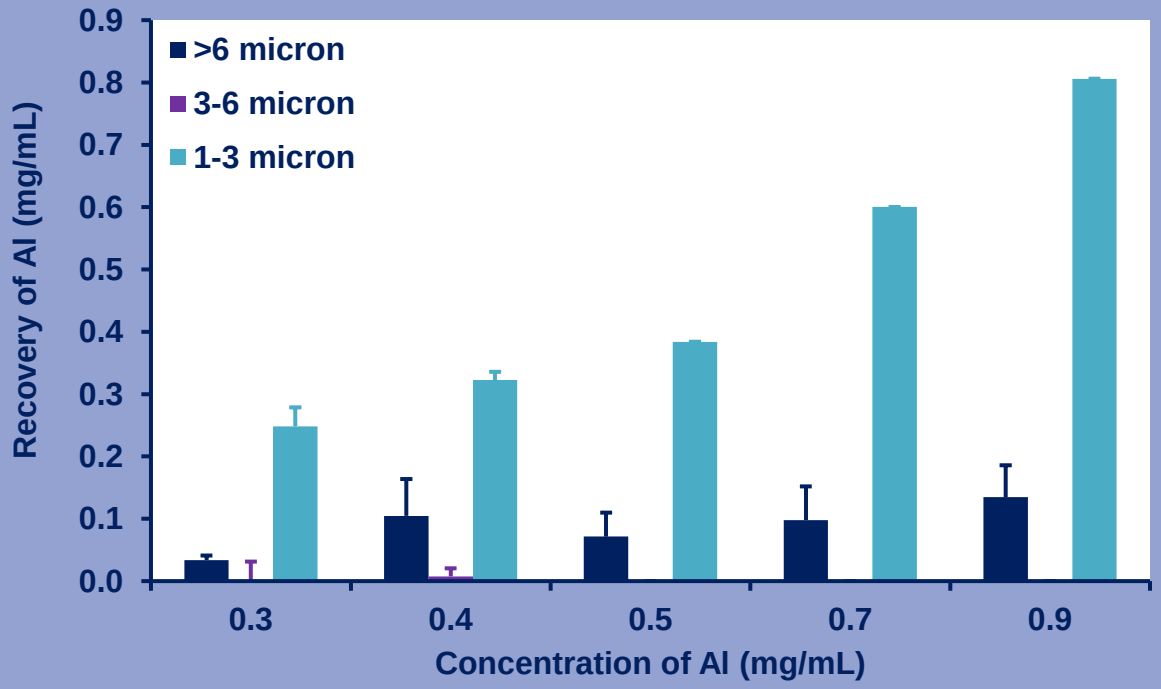
# ELS – Zeta potential vs. concentration of Al in simulated vaccines.




- Zeta potential values remained fairly consistent over the concentration range studied and were located in the region associated with systemic instability (-11.52- -12.28mV).
- No significant difference between concentrations of Al
- Vaccine particles are negatively charged when administered into R10 medium.
- Evidence of protein adsorption (surface saturation) at all concentrations studied.

***Fig 9: Zeta potential of Alhydrogel only vaccines containing 0.3-0.9mg/mL Al following 1hr incubation within R10 medium (37°C). Error bars represent the ±SD of the measurement where n=5***

# GFAAS/filtration – % aluminium recovery of simulated vaccines in R10 medium (1hr incubation)

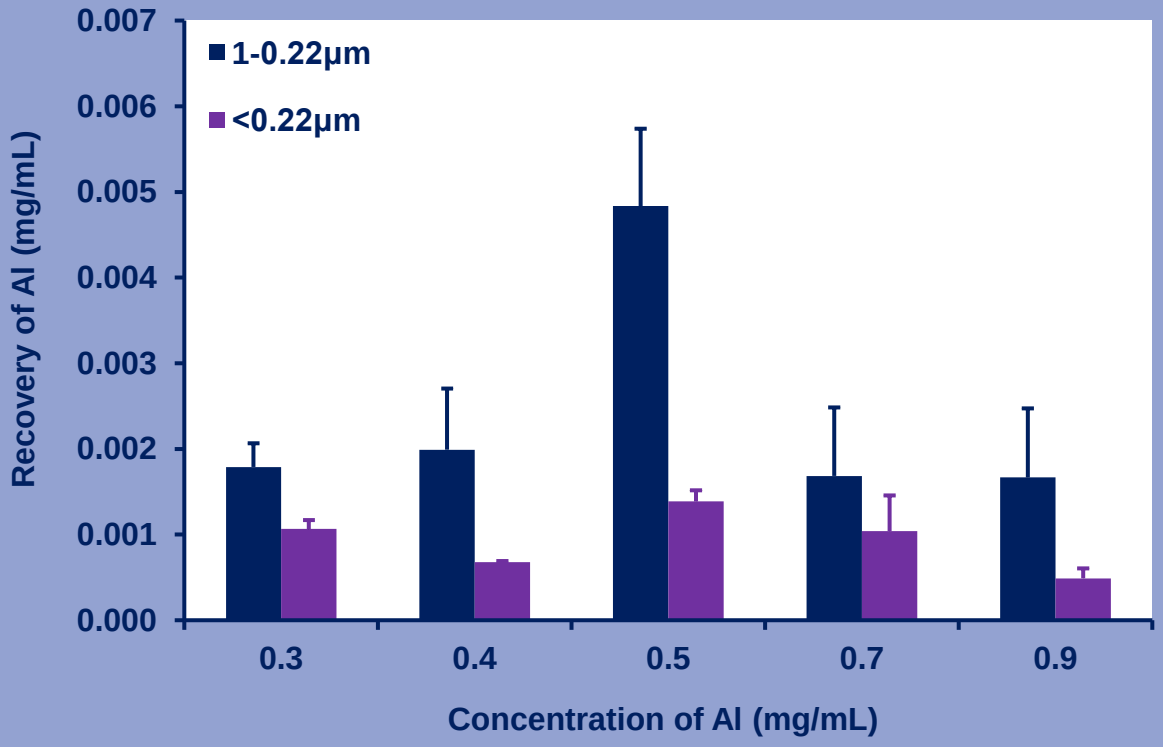



| Concentration of Al (mg/mL) | 0.3 | 0.4 | 0.5 | 0.7 | 0.9 |
|---|---|---|---|---|---|
| | Recovery of Al (%) | | | | |
| 1-3µm | 87.43 | 73.56 | 83.46 | 85.99 | 85.69 |
| 3-6µm | 0.18 | 1.69 | 0 | 0 | 0 |
| >6µm | 11.78 | 23.80 | 15.48 | 14.01 | 14.31 |

- Abundance of particles between 1-3µm increased when concentration of Al increased.
- Significant differences observed between:
  - ❖ 0.3mg/mL & 0.7mg/mL (0.25mg/mL vs. 0.6 mg/mL, P=0.01)
  - ❖ 0.3mg/mL & 0.9mg/mL (0.25mg/mL vs. 0.8 mg/mL, P=0.0001)
  - ❖0.4 mg/mL & 0.9 mg/mL (0.32 mg/mL vs. 0.8mg/mL, P=0.01
- Larger availability of smaller particles at higher concentrations of Al

***Fig 10: The relative abundance of aluminium (%) within specific particle size fractions for Alhydrogel only vaccines containing an initial concentration of 0.3-0.9mg/mL Al following 1 hr incubation within R10 medium (37°C). Blue, purple and green boxes represent the % Al in the size fractions >6µm, 3-6µm & 1-3µm respectively.***

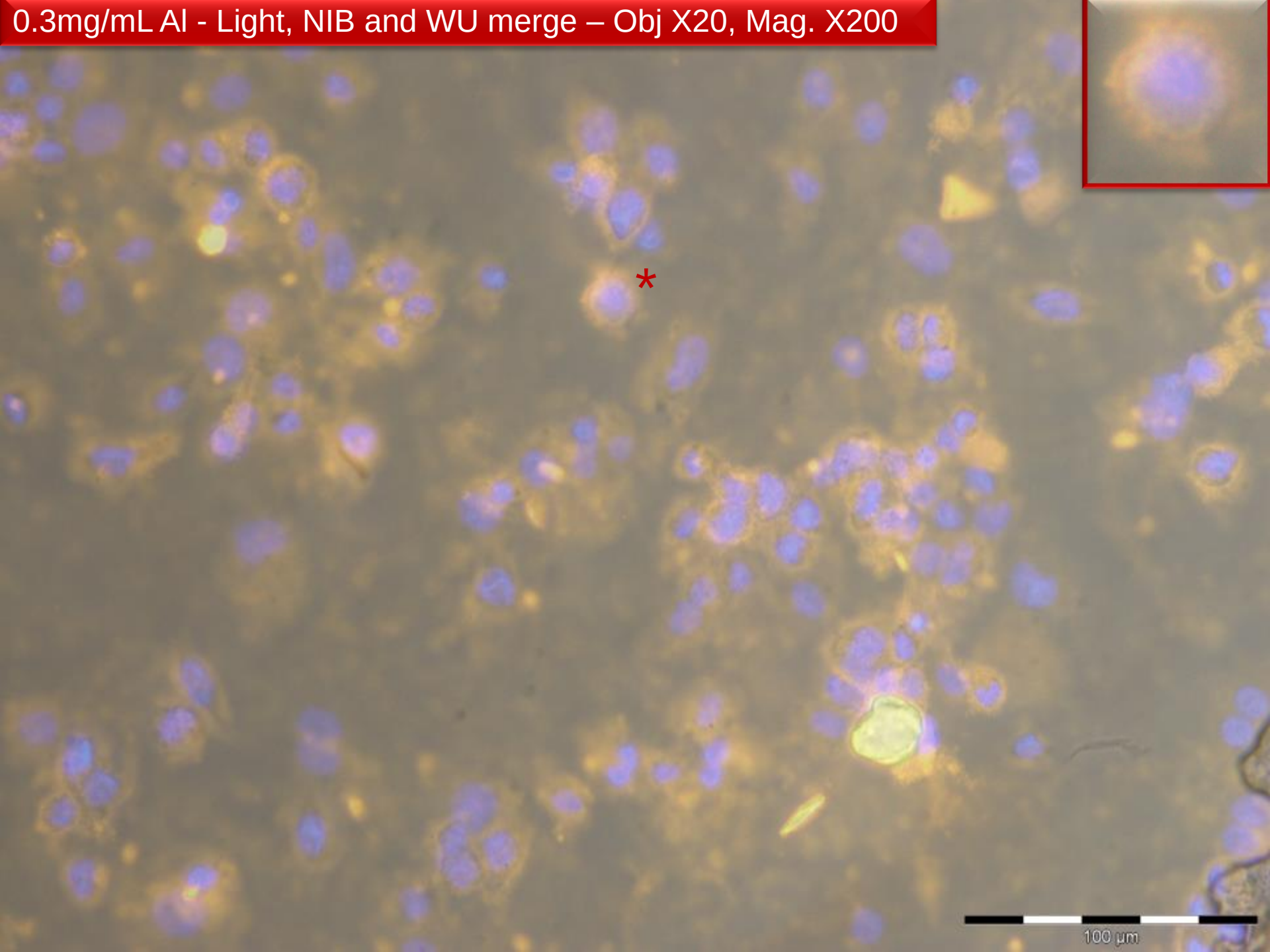
# GFAAS/filtration – % aluminium recovery of simulated vaccines in R10 medium (1hr incubation)



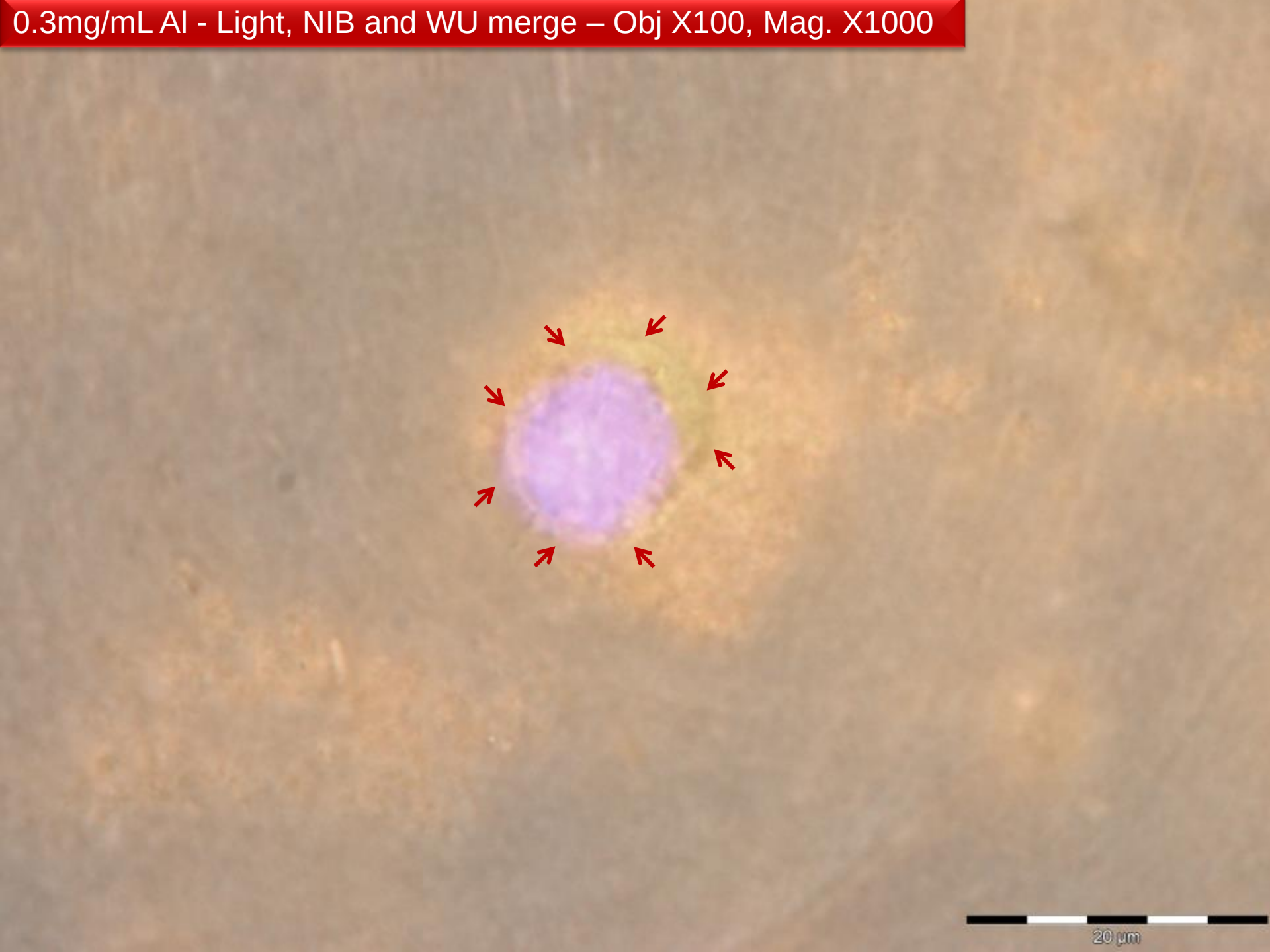

- Abundance of particles <0.22 µm remained consistent when concentration of Al increased.
- Significant differences observed between:
  - 0.5mg/mL & 0.9mg/mL (0.001 mg/mL vs. 0.0002 mg/mL, P=0.004)

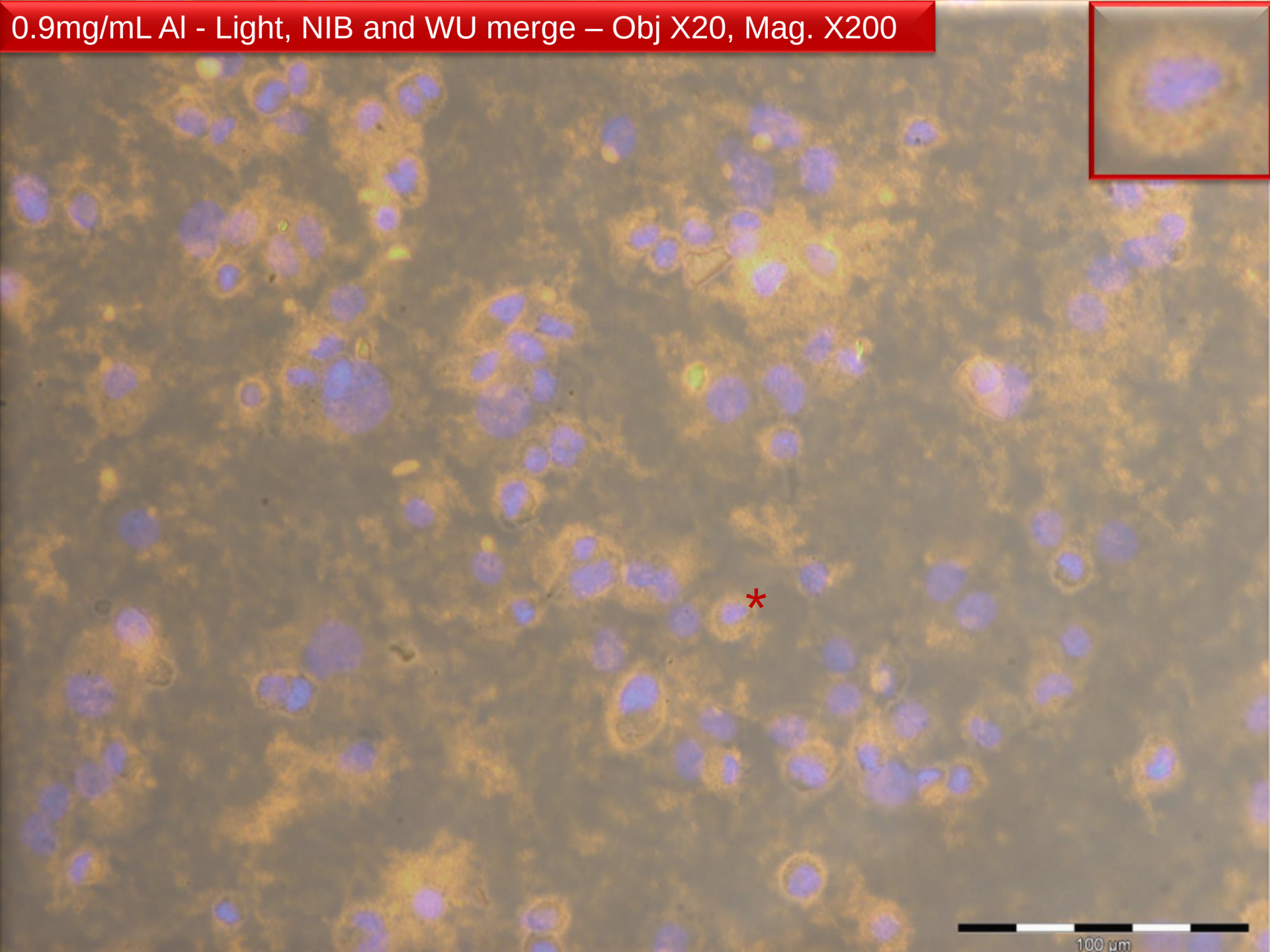
| Concentration of Al (mg/mL) | 0.3 | 0.4 | 0.5 | 0.7 | 0.9 |
|---|---|---|---|---|---|
| | Recovery of Al (%) | | | | |
| 1-0.22µm | 0.630 | 0.454 | 1.051 | 0.241 | 0.177 |
| <0.22µm | 0.375 | 0.154 | 0.302 | 0.149 | 0.052 |

***Fig 11: The relative abundance of aluminium (%) within specific particle size fractions for Alhydrogel only vaccines containing an initial concentration of 0.3-0.9mg/mL Al following 1 hr incubation within R10 medium (37°C). Blue & purple boxes represent the % Al in the size fractions 1-0.22µm & >0.22µm respectively.***


0.3mg/mL Al - Light, NIB and WU merge – Obj X20, Mag. X200
100 µm

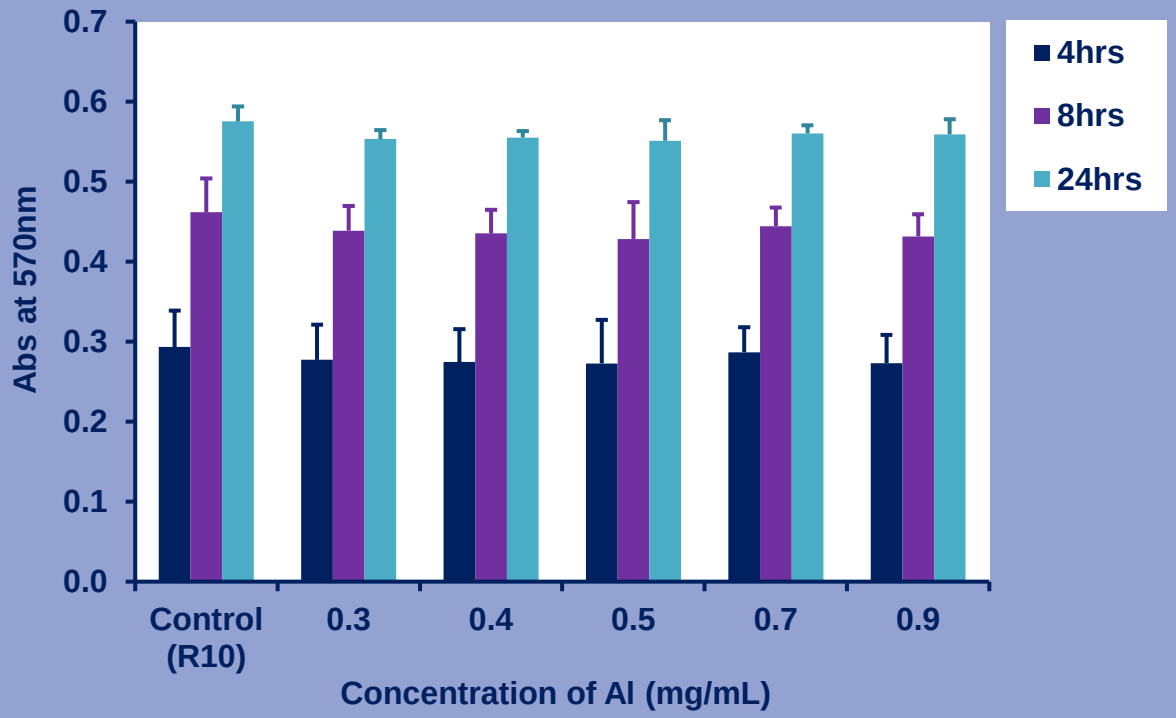
# 0.3mg/mL Al - Light, NIB and WU merge – Obj X100, Mag. X1000




0.9mg/mL Al - Light, NIB and WU merge – Obj X20, Mag. X200
*
100 µm


0.9mg/mL Al - Light and WU merge – Obj X100, Mag. X1000
20 µm

# Presto blue- Viability of macrophages exposed to various concentrations of Al in simulated vaccines.

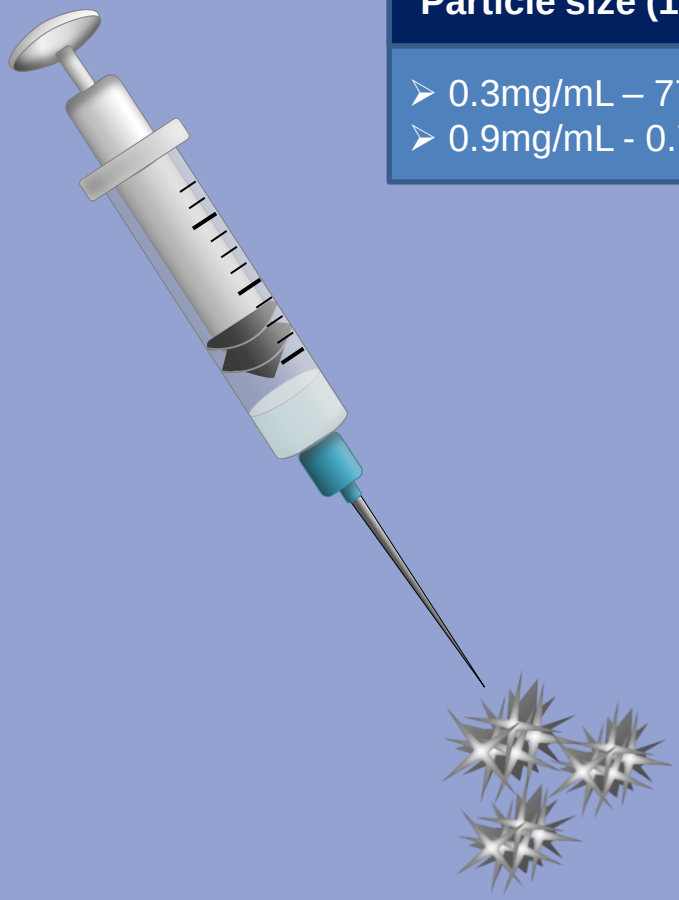



- Null significance between treatment values and control at all time points.
- Cell viability was unaffected by all exposure regimes post 24hrs.

| Concentration of Al (mg/mL) | 0.3 | 0.4 | 0.5 | 0.7 | 0.9 |
|---|---|---|---|---|---|
| Time | % Control | | | | |
| 4hrs | 94.6 | 93.6 | 93.0 | 97.8 | 93.2 |
| 8hrs | 95.0 | 94.3 | 92.8 | 96.2 | 93.5 |
| 24hrs | 96.2 | 96.5 | 95.8 | 97.4 | 97.2 |

*Fig 12: The viability of macrophages exposed to various concentrations of Al over a total duration of 24hrs. Error bars represent the ±SD of the measurement where n=5.*

# Conclusions



**Particle size (1-3µm)**

- 0.3mg/mL – 77%
- 0.9mg/mL - 0.7%

**Particle size in R10 (1-3µm)**

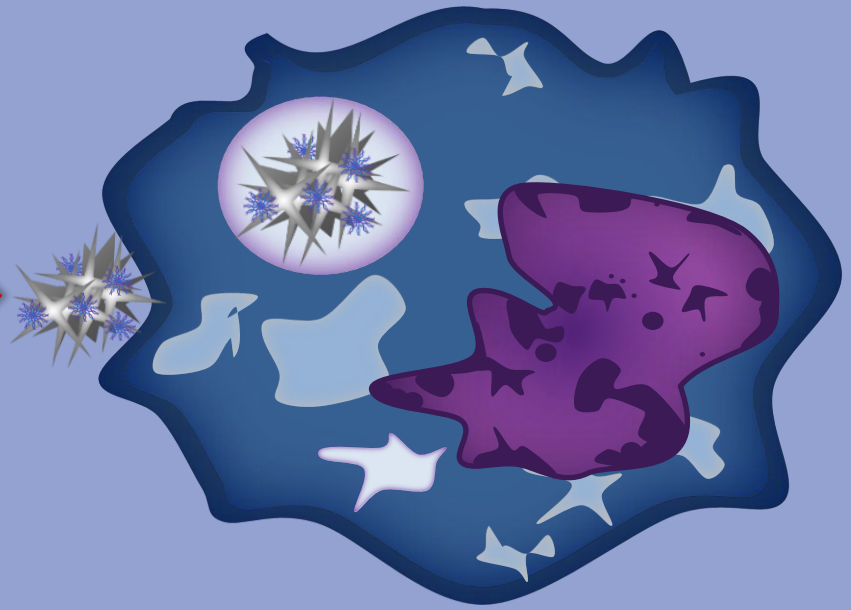
- 0.3mg/mL – 87%
- 0.9mg/mL - 85%

**Uptake**

- Uptake observed at low and high concentrations Al.

**Cell viability**

- Post uptake macrophage survival was not impaired following 24hrs incubation.
- Translocation to lymph nodes highly likely at all concentrations studied.



# References


[1] Baylor NW, Egan W, Richman P. Aluminium salts in vaccines – US perspective. *Vaccine*, 2002, 29:S18-23.

[2] Majgaard Jensen O, Koch C. On the effect of Al(OH)3 as an immunological adjuvant. APMIS,1988, 96(3):257-64.

[3] Gupta RK, Rost BE, Relyveld E, Siber GR. Adjuvant properties of aluminium and calcium compounds. In: Powell, M.F. Newman, M.J. (eds.) *Vaccine Design: The Subunit and Adjuvant Approach.* 1995 . New York, US: Plenum.

# Acknowledgements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CMSRI
CHILDREN'S MEDICAL SAFETY RESEARCH INSTITUTE

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