# A Commercial In-Vitro Biological Activity Potency Assay for AAV-AQP1 Treatment of Xerostomia

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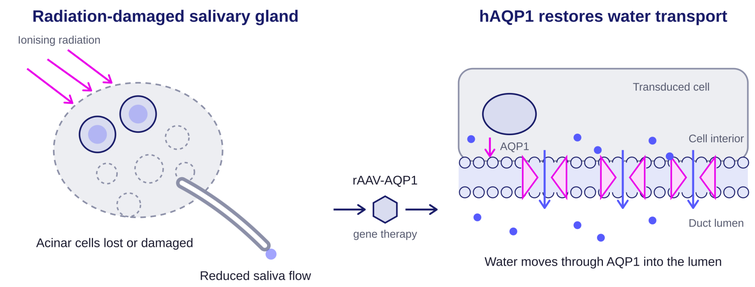
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## 1. Introduction

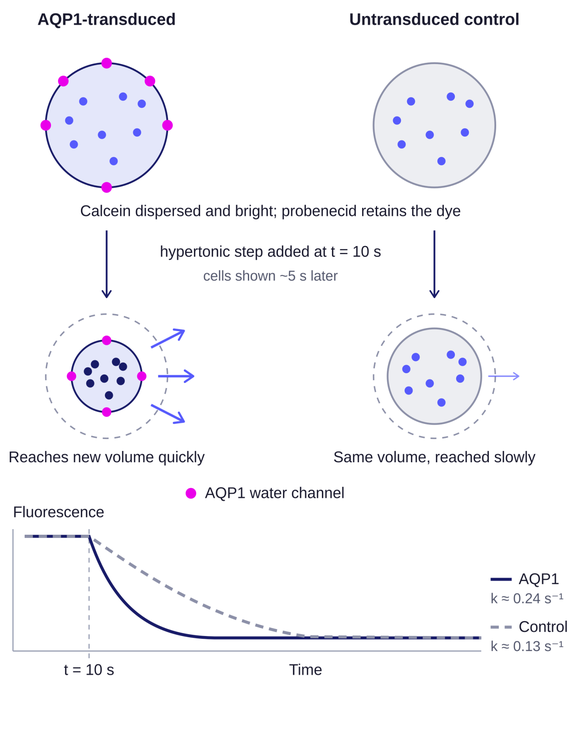
Xerostomia following ionising radiation for head and neck cancer, or arising from Sjögren's syndrome, causes irreversible salivary gland damage, oral dryness, impaired chewing and swallowing, and reduced quality of life.

MeiraGTx's rAAV-AQP1 gene therapy restores localised water movement in damaged glands by expressing human aquaporin 1, a constitutively active membrane water channel driving osmotic water transport.

Due to the mode of action being water transport, vector genome titre and transgene expression do not on their own demonstrate function. A potency assay reflecting the mode of action is required for QC release, stability testing and commercial licensure.




## 2. Assay Principle



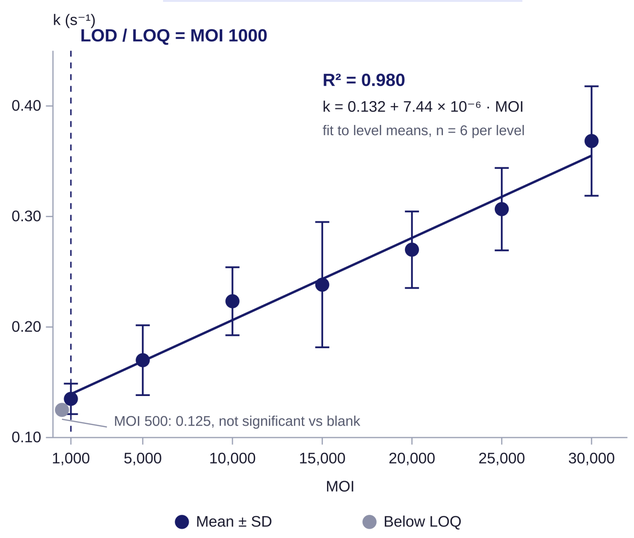

Calcein-loaded cells are challenged with a hyperosmotic sugar gradient. Water leaves the cell, the cell shrinks, intracellular calcein concentrates above its self-quenching threshold and fluorescence falls. The rate of that fall reports osmotic water permeability, and therefore functional AQP1. Typical k ≈ 0.24 $s^{-1}$ transduced and ≈ 0.13 $s^{-1}$ untransduced.

T98G cells were selected over HeLa for lower endogenous AQP1 activity, a broader linear range and compatibility with complementary expression studies. Cells are seeded at 5 × $10^4$ cells/mL, transduced with rAAV-AQP1 for 48 h and loaded with calcein-AM in medium containing 1 mM probenecid, which inhibits organic-anion transporter–mediated dye efflux and holds the intracellular dye pool stable across the read.

Fluorescence is read for 10 s to establish baseline, the challenge is injected at the 10 s mark and the trace acquired for 50 s. Data are fitted in GraphPad Prism to a plateau followed by one-phase decay with $X_0$ constrained to the injection time, returning the observed rate constant k.

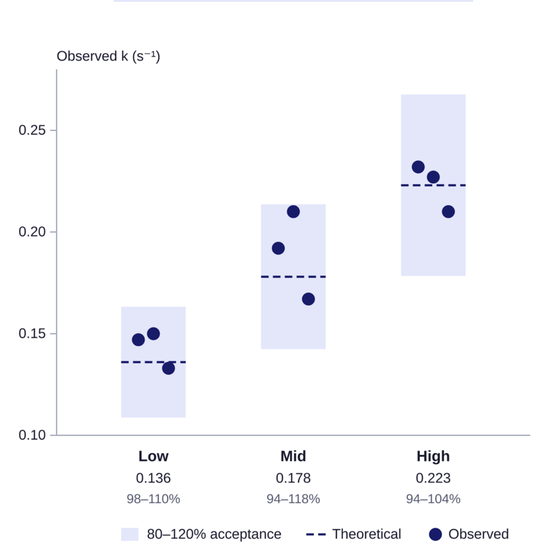
## 3. Development and Validation Results

### Linearity



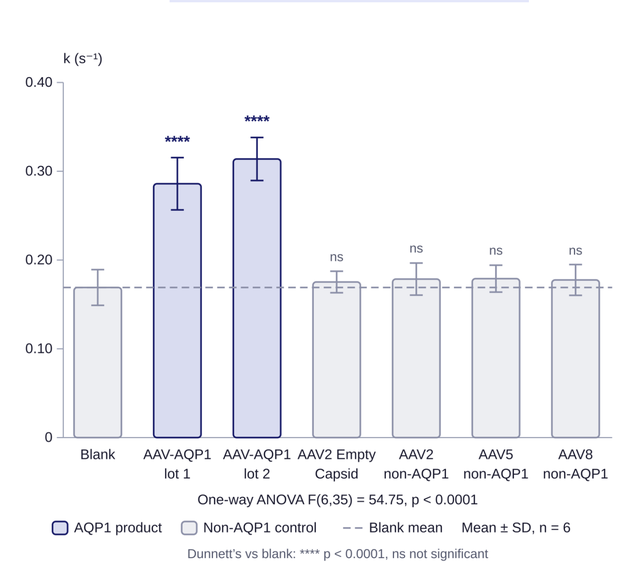

Mean k against MOI, six replicates per level. R² = 0.980 over MOI 1,000–30,000. MOI 1,000 is the LOD and LOQ: significant against blank (p = 0.0030), 9.4% CV and 98–110% recovery. MOI 500 (grey) is not significant against blank.

### Accuracy



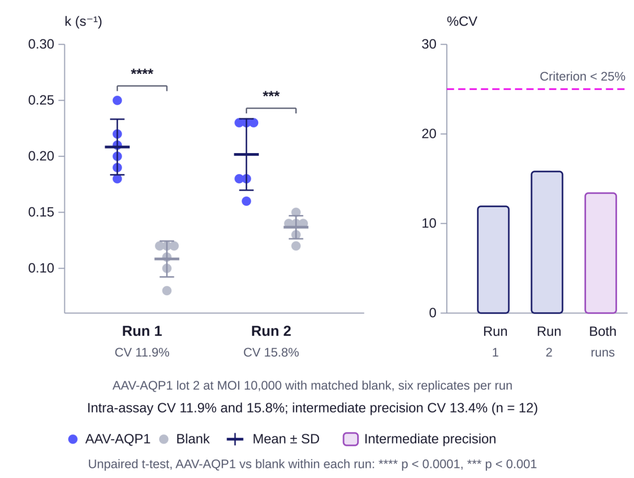

Observed against theoretical k at three levels spanning the range, three replicates each. Shaded bands show the 80–120% recovery criterion. All nine replicates are within specification, with recoveries of 98–110%, 94–118% and 94–104%.

### Specificity



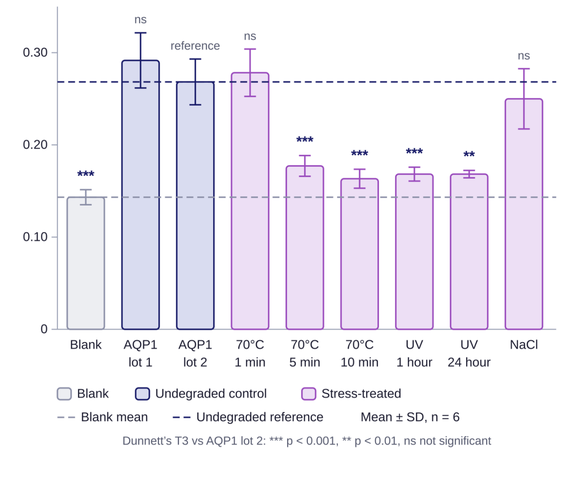

Two rAAV-AQP1 products against four non-AQP1 controls, six replicates each. Both AQP1 products differ from blank at p < 0.0001, while empty capsid, AAV2 non-AQP1, AAV5 and AAV8 are indistinguishable from blank (adjusted p = 0.899–0.988).

### Precision




Six replicates of AAV-AQP1 lot 2 at MOI 10,000 per analyst, run on different days, equipment and passage numbers. Intra-assay CV was 11.9% and 15.8%. Intermediate precision was 13.4%, within the <25% criterion, and reflects the offset between analyst means rather than scatter within either.

### Stability-Indicating




Forced degradation against the undegraded AAV-AQP1 lot 2 reference. Heat at 70 °C gives a time-dependent loss of activity: 1 min is indistinguishable from undegraded, 5 and 10 min are significantly reduced. Both UV arms are reduced. NaCl did not degrade the material (p = 0.9027).

### Robustness




Independent development runs grouped three ways. No comparison is significant: operators p = 0.3034 (n = 24), injectors p = 0.6408 (n = 25), seed trains p = 0.1266 (n = 27). Operator means of 0.196 and 0.211 across 24 runs show no systematic operator effect.

## 4. Summary

| Characteristic | Criterion | Result | Outcome |
|---|---|---|---|
| Linearity | $R^2 \geq 0.96$ | $R^2 = 0.98$, MOI 1,000–30,000 | Pass |
| Range | — | MOI 1,000–30,000 | Pass |
| Accuracy | 80–120% recovery | 94–118% | Pass |
| Intra-assay precision | %CV < 25.0% | 11.9% and 15.8% | Pass |
| Intermediate precision | %CV < 25.0% | 13.4% | Pass |
| Specificity | No interference | 4 / 4 non-AQP1 controls ns | Pass |
| LOD / LOQ | Significant vs blank | MOI 1,000 | Pass |
| Robustness | ±20% | Operator, injector, seed train, passage all ns | Pass |
| Stability-indicating | Detects degradation | Loss at 70 °C; UV degraded | Pass |

## 5. Conclusion

- Potency is measured through the mode of action, water transport, rather than a surrogate for expression.
- Validated to ICH Q2(R2); every predefined acceptance criterion was met.
- Specificity shown against empty capsid, matched serotype and other serotypes.
- Stability-indicating, resolving graded loss of activity with increasing thermal stress.
- Robust to operator, instrument, seed train and passage.
- Supports product development, QC release and future commercial licensure.