A NOVEL STEM CELL POTENCY and RELEASE ASSAY for UMBILICAL CORD BLOOD.

Correlation of Stem Cell Proliferation with Engraftment


ABSTRACT

Acceptance or rejection of an umbilical cord blood (UCB) unit is determined by total nucleated cell count (TNC). For UCB, stem cell potency will decide whether a UCB unit will engraft and repopulate the recipient without rejection following transplantation. A cell potency and release assay is required to monitor stem cell potency and reliability. The HALO® ATP assay, a novel instrument-based, ATP bioluminescence proliferation assay, was used to validate and compare at two geographical locations. Results indicated a correlation of -0.99 in the assay between the UCB and the standard UCB reference concentration between days 1 and 14. The ATP assay has now been further developed into a stem cell potency and release assay using UCB reference standard. This assay is an alternative to colony-forming cell (CFC) assay for evaluating stem cell potency. A cell potency and release assay can help to establish the patient engraftment and the potential for malignancy following transplantation. The HALO® ATP assay can be used to evaluate different lineages of transplant stem cell products, and their lineagespecific dependency on ATP (CFC-GM, BFU-E) by testing stem cells from UCB units.

WHY IS A CELL POTENCY ASSAY SO IMPORTANT?

1. To control for batch manufacturing consistency.
2. Product stability.
3. Product bridging studies.
4. Controls for pipetting error and bad technique.
5. Helps validate the assay

REGULATORY ISSUES

International Guidelines for Stem Cell Standards

According to AABB Standards for Cellular Therapy Product Service:
Section D14.1.1.1 states, “The use of established and validated appropriate assays, standards, and controls for the evaluation of clinical safety, purity, potency and identity of cellular products is required. Adequate provisions for monitoring the reliability, accuracy, precision and robustness of these tests is also required.”
Section D14.1.1.2 states, “Adequate provisions for monitoring the reliability, accuracy, precision and robustness of these tests is also required.”

According to FACT-JACIE International Standards, 3rd Edition:
Section D6.13 states, “Laboratory processes shall include the establishment of appropriate and validated assays, test procedures, the evaluation of test accuracy, and the use of quality controls for monitoring the reliability, accuracy, precision and robustness of these tests.”

According to NetCord-FACT Standards for Cord Blood, 3rd Edition:
“Laboratory processes shall include the establishment of appropriate and validated assays, test procedures, the evaluation of test accuracy, and the use of quality controls for monitoring the reliability, accuracy, precision and robustness of these tests.”

Correlation of Colony Number with Engraftment

- Three independent units of cord blood did exhibit engraftment, despite the fact that the ATP concentrations were significantly lower than the acceptance limit.
- Three samples that were not predicted to have engrafted, apparently did engraft. All other samples that were below the acceptance limit, did not engraft.

CONCLUSIONS

HALO®-96 PQR is the only in vitro assay available that can:
- Reliably measure and screen for high and low stem cell potency against a reference standard.
- Define acceptance limits for engraftment of cord blood units for stem cell transplantation.
- Predict cord blood transplantation outcome.

Despite the standardized use of “acceptable” and “validated” assays to detect and measure cell potency, no standardized method for measurement of cord blood potency by Standards Organizations and Regulatory Agencies for cord blood transplantation can be used. The HALO® ATP assay has been validated many times not only for the CFC assay, but also for the Registry of Cytotoxicity Prediction Model as a validated approach by the Cord Blood Biologics Standards Organizations. The CALC assay is currently under validation for use in patient-related clinical applications.

The HALO®-96 Stem and Progenitor Cell Quality Control (CFC-QC) and HALO®-96 Potency, Quality, Release. Platforms have been specifically developed to assess stem cell potency and cord blood processing laboratories. The HALO® Platform has been validated many times not only against the CFC assay, but also against the Registry of Cytotoxicity Prediction Model as a validated approach by the Cord Blood Biologics Standards Organizations and companies that new consider HALO® the “GOLD STANDARD” in cord testing.

Further studies are ongoing with fresh and frozen cord blood samples. It is expected that future results will considerably change and correct these reported findings. In the future, researchers and clinicians will rely on this assay to assess, engraftment and predict patient outcome.

The HALO®-96 PQR assay is an alternative to CFC and TK assays. In this assay, ATP bioluminescence is measured in a plate luminometer as luminescence at RT in 10 min prior to sample measurement. The ATP assay is an alternative to colony-forming cell (CFC) assay for evaluating stem cell potency. The HALO® ATP assay can be used to evaluate different lineages of transplant stem cell products, and their lineagespecific dependency on ATP (CFC-GM, BFU-E) by testing stem cells from UCB units.

WHY IS A CELL POTENCY ASSAY SO IMPORTANT?

1. To control for batch manufacturing consistency.
2. Product stability.
3. Product bridging studies.
4. Controls for pipetting error and bad technique.
5. Helps validate the assay

REGULATORY ISSUES

International Guidelines for Stem Cell Standards

According to AABB Standards for Cellular Therapy Product Service:
Section D14.1.1.1 states, “The use of established and validated appropriate assays, standards, and controls for the evaluation of clinical safety, purity, potency and identity of cellular products is required. Adequate provisions for monitoring the reliability, accuracy, precision and robustness of these tests is also required.”
Section D14.1.1.2 states, “Adequate provisions for monitoring the reliability, accuracy, precision and robustness of these tests is also required.”

According to FACT-JACIE International Standards, 3rd Edition:
Section D6.13 states, “Laboratory processes shall include the establishment of appropriate and validated assays, test procedures, the evaluation of test accuracy, and the use of quality controls for monitoring the reliability, accuracy, precision and robustness of these tests.”

According to NetCord-FACT Standards for Cord Blood, 3rd Edition:
“Laboratory processes shall include the establishment of appropriate and validated assays, test procedures, the evaluation of test accuracy, and the use of quality controls for monitoring the reliability, accuracy, precision and robustness of these tests.”

Correlation of Colony Number with Engraftment

- Three independent units of cord blood did exhibit engraftment, despite the fact that the ATP concentrations were significantly lower than the acceptance limit.
- Three samples that were not predicted to have engrafted, apparently did engraft. All other samples that were below the acceptance limit, did not engraft.

CONCLUSIONS

HALO®-96 PQR is the only in vitro assay available that can:
- Reliably measure and screen for high and low stem cell potency against a reference standard.
- Define acceptance limits for engraftment of cord blood units for stem cell transplantation.
- Predict cord blood transplantation outcome.

Despite the standardized use of “acceptable” and “validated” assays to detect and measure cell potency, no standardized method for measurement of cord blood potency by Standards Organizations and Regulatory Agencies for cord blood transplantation can be used. The HALO® ATP assay has been validated many times not only for the CFC assay, but also against the Registry of Cytotoxicity Prediction Model as a validated approach by the Cord Blood Biologics Standards Organizations. The CALC assay is currently under validation for use in patient-related clinical applications.

The HALO®-96 Stem and Progenitor Cell Quality Control (CFC-QC) and HALO®-96 Potency, Quality, Release. Platforms have been specifically developed to assess stem cell potency and cord blood processing laboratories. The HALO® Platform has been validated many times not only against the CFC assay, but also against the Registry of Cytotoxicity Prediction Model as a validated approach by the Cord Blood Biologics Standards Organizations and companies that new consider HALO® the “GOLD STANDARD” in cord testing.

Further studies are ongoing with fresh and frozen cord blood samples. It is expected that future results will considerably change and correct these reported findings. In the future, researchers and clinicians will rely on this assay to assess, engraftment and predict patient outcome.

The HALO®-96 PQR assay is an alternative to CFC and TK assays. In this assay, ATP bioluminescence is measured in a plate luminometer as luminescence at RT in 10 min prior to sample measurement. The ATP assay is an alternative to colony-forming cell (CFC) assay for evaluating stem cell potency. The HALO® ATP assay can be used to evaluate different lineages of transplant stem cell products, and their lineagespecific dependency on ATP (CFC-GM, BFU-E) by testing stem cells from UCB units.

WHY IS A CELL POTENCY ASSAY SO IMPORTANT?

1. To control for batch manufacturing consistency.
2. Product stability.
3. Product bridging studies.
4. Controls for pipetting error and bad technique.
5. Helps validate the assay

REGULATORY ISSUES

International Guidelines for Stem Cell Standards

According to AABB Standards for Cellular Therapy Product Service:
Section D14.1.1.1 states, “The use of established and validated appropriate assays, standards, and controls for the evaluation of clinical safety, purity, potency and identity of cellular products is required. Adequate provisions for monitoring the reliability, accuracy, precision and robustness of these tests is also required.”
Section D14.1.1.2 states, “Adequate provisions for monitoring the reliability, accuracy, precision and robustness of these tests is also required.”

According to FACT-JACIE International Standards, 3rd Edition:
Section D6.13 states, “Laboratory processes shall include the establishment of appropriate and validated assays, test procedures, the evaluation of test accuracy, and the use of quality controls for monitoring the reliability, accuracy, precision and robustness of these tests.”

According to NetCord-FACT Standards for Cord Blood, 3rd Edition:
“Laboratory processes shall include the establishment of appropriate and validated assays, test procedures, the evaluation of test accuracy, and the use of quality controls for monitoring the reliability, accuracy, precision and robustness of these tests.”

Correlation of Colony Number with Engraftment

- Three independent units of cord blood did exhibit engraftment, despite the fact that the ATP concentrations were significantly lower than the acceptance limit.
- Three samples that were not predicted to have engrafted, apparently did engraft. All other samples that were below the acceptance limit, did not engraft.

CONCLUSIONS

HALO®-96 PQR is the only in vitro assay available that can:
- Reliably measure and screen for high and low stem cell potency against a reference standard.
- Define acceptance limits for engraftment of cord blood units for stem cell transplantation.
- Predict cord blood transplantation outcome.

Despite the standardized use of “acceptable” and “validated” assays to detect and measure cell potency, no standardized method for measurement of cord blood potency by Standards Organizations and Regulatory Agencies for cord blood transplantation can be used. The HALO® ATP assay has been validated many times not only for the CFC assay, but also against the Registry of Cytotoxicity Prediction Model as a validated approach by the Cord Blood Biologics Standards Organizations and companies that new consider HALO® the “GOLD STANDARD” in cord testing.

Further studies are ongoing with fresh and frozen cord blood samples. It is expected that future results will considerably change and correct these reported findings. In the future, researchers and clinicians will rely on this assay to assess, engraftment and predict patient outcome.

The HALO®-96 PQR assay is an alternative to CFC and TK assays. In this assay, ATP bioluminescence is measured in a plate luminometer as luminescence at RT in 10 min prior to sample measurement. The ATP assay is an alternative to colony-forming cell (CFC) assay for evaluating stem cell potency. The HALO® ATP assay can be used to evaluate different lineages of transplant stem cell products, and their lineagespecific dependency on ATP (CFC-GM, BFU-E) by testing stem cells from UCB units.