The BlueScreen HC™ Assay to Predict the Genotoxic Potential for Fragrance Materials

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Abstract

BlueScreen HC™ is a mammalian cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. In this assay a genetically modified strain of cultured human lymphoblastoid TK6 cells is used (GLuc-T01). Incorporated into this strain is a patented Gaussia luciferase (GLuc) reporter system that exploits the regulation of the GADD45a gene, which mediates the adaptive response to genotoxic stress. BlueScreen HC™ has been utilized at the Research Institute for Fragrance Materials (RIFM) in a safety assessment program as a screening tool to prioritize materials for testing, as supporting evidence when using a read-across approach, and as evidence to adjust the threshold of toxicological concern (TTC). Predictive values (positive and negative) for the BlueScreen HC™ assay were calculated based on the ability of the assay to conclude whether the materials are genotoxic or non-genotoxic, determined by the outcome in regulatory approved assays. A set of 379 fragrance materials were assessed in the BlueScreen HC™ assay along with regulatory approved assays conducted in compliance with GLP regulations and in accordance with appropriate OECD guidelines. BlueScreen HC™ results were highly predictive of positive and negative outcome from regulatory compliant genotoxicity assays for this set of fragrance materials. The BlueScreen HC™ negative results for genotoxicity were in 100% alignment with negative results obtained in regulatory compliant genotoxicity assays. In addition, fragrance materials which exhibited a positive result in the BlueScreen HC™ assay were in 100% alignment with positive results in regulatory compliant genotoxicity assays. Based on this analysis the BlueScreen HC™ assay is a useful high throughput screening approach in the fragrance safety assessment program.

Introduction

Regulatory approved in vitro genotoxicity assays based on prokaryotic and eukaryotic systems have been of great importance in order to evaluate genotoxic potential of materials. These assays have been of much more importance in recent times due to an initiative to develop animal alternative testing strategies, however the only drawback of these test systems are they are time consuming and labor intensive when it comes to evaluating multiple chemicals at a time. This has led to an effort to develop mammalian based high through put screening assays (HTS) which can screen large number of materials at a single point of time. HTS may also help to identify mechanism of action on a molecular level. High throughput screening tools to identify genotoxic chemicals are an essential step to screen potential carcinogens at a preliminary stage. BlueScreen HC™ is one of the HTS assays which could be a helpful tool to ensure safety of fragrance chemicals. BlueScreen HC™ is a mammalian cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures.

Materials and Methods

BlueScreen HC assay: BlueScreen HC™ is a mammalian cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. In this assay, a genetically modified strain of cultured human lymphoblastoid TK6 cells is used (GLuc-T01). Incorporated into this strain is a patented Gaussia luciferase (GLuc) reporter system that exploits the proper regulation of the GADD45a gene, which mediates the adaptive response to genotoxic stress. Exposure to a genotoxic material increases expression of GLuc, which is quantified at the assay endpoint by the detection of luminescence generated from the reaction of GLuc with a coelenterazine substrate, added to the microplate wells just before measurement.
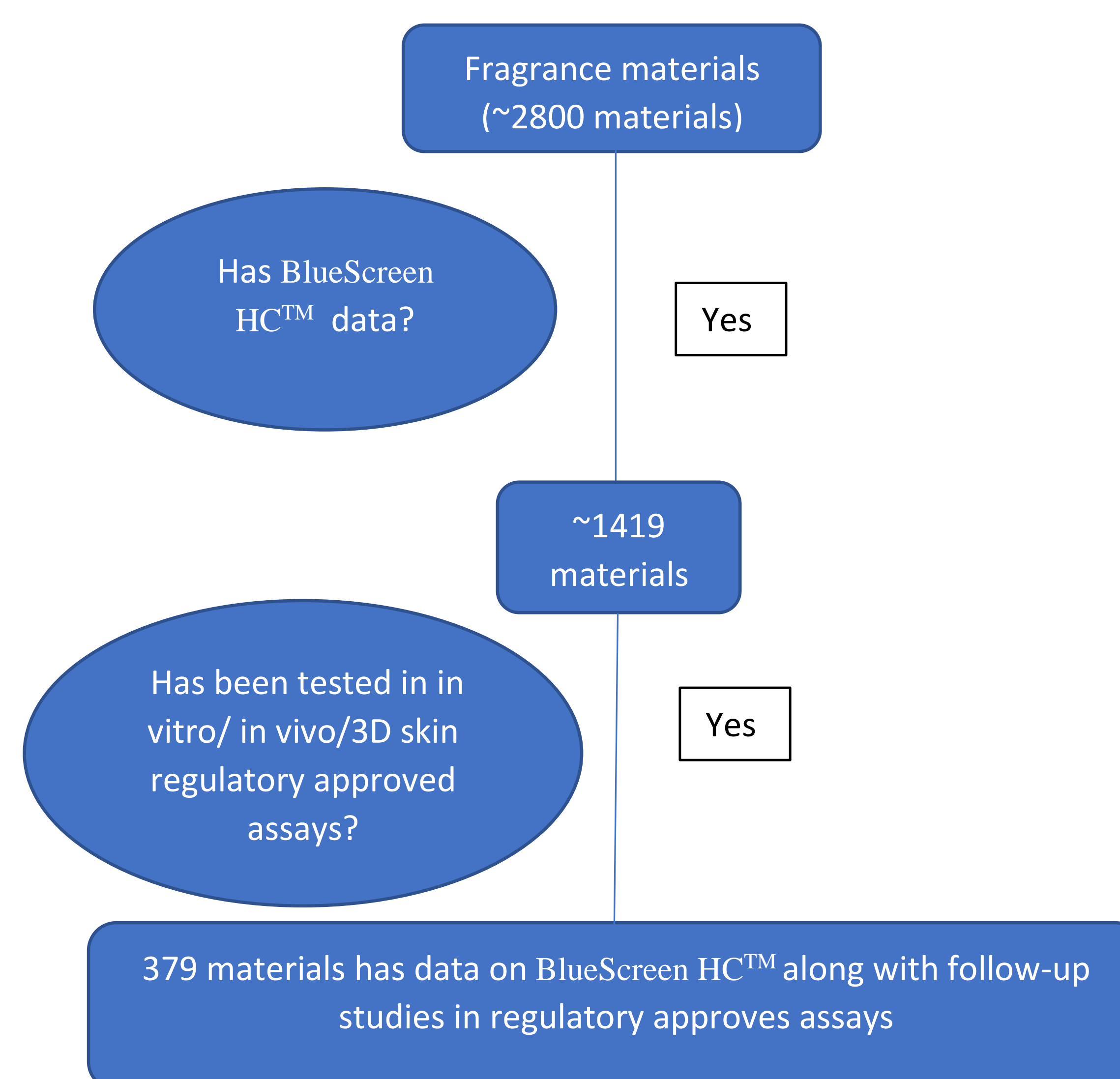
Selection of Materials

Fig. 1. Criteria for selection of Materials

Data Analysis

$$\text{Positive agreement} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}}$$

$$\text{Negative agreement} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Positive}}$$
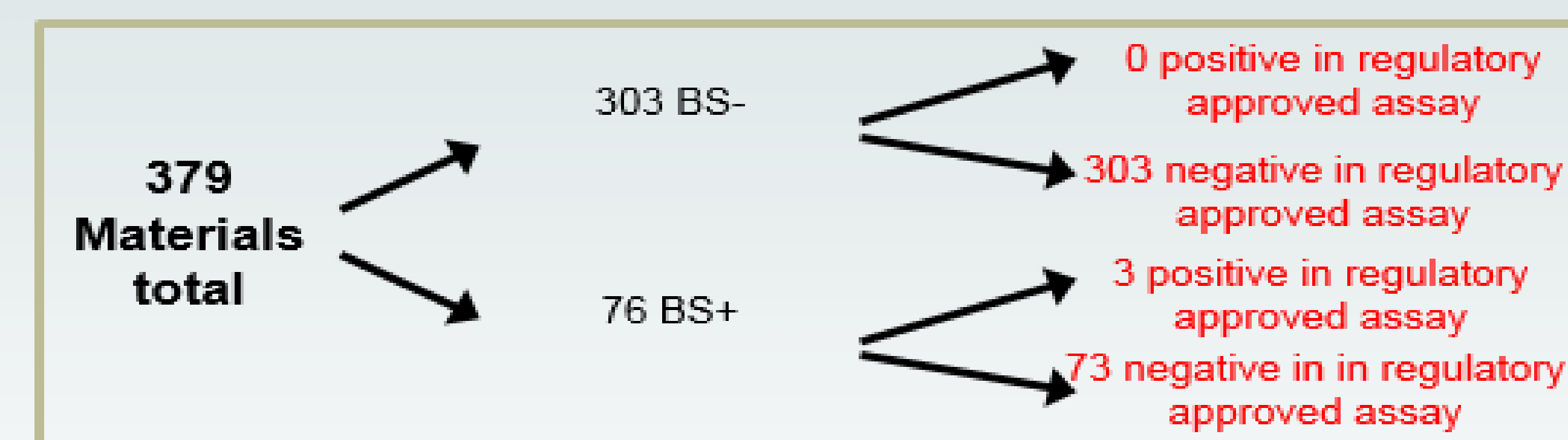
$$\text{Predictivity (Positive)} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}}$$

$$\text{Predictivity (Negative)} = \frac{\text{True Negative}}{\text{True Negaive} + \text{False Negative}}$$
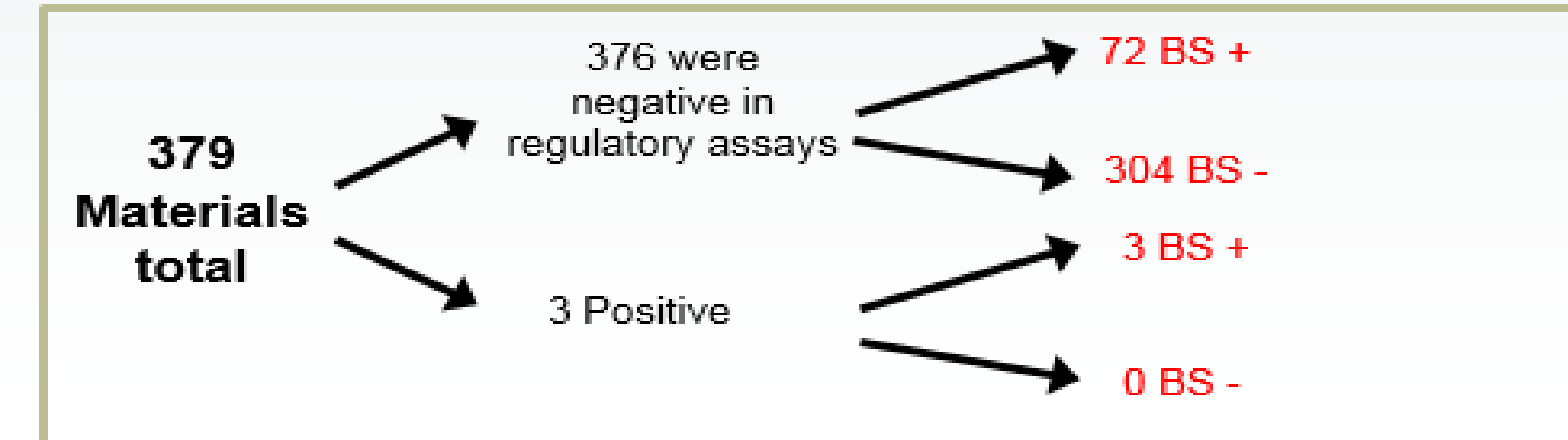
Results

Predictive and Agreement Analysis

Fig. 2. Bluescreen Analysis: Predictive and Agreement

Performance Parameters

Positive Predictive Value: 4%

Negative Predictive Value: 100%

Positive Agreement Value: 100%
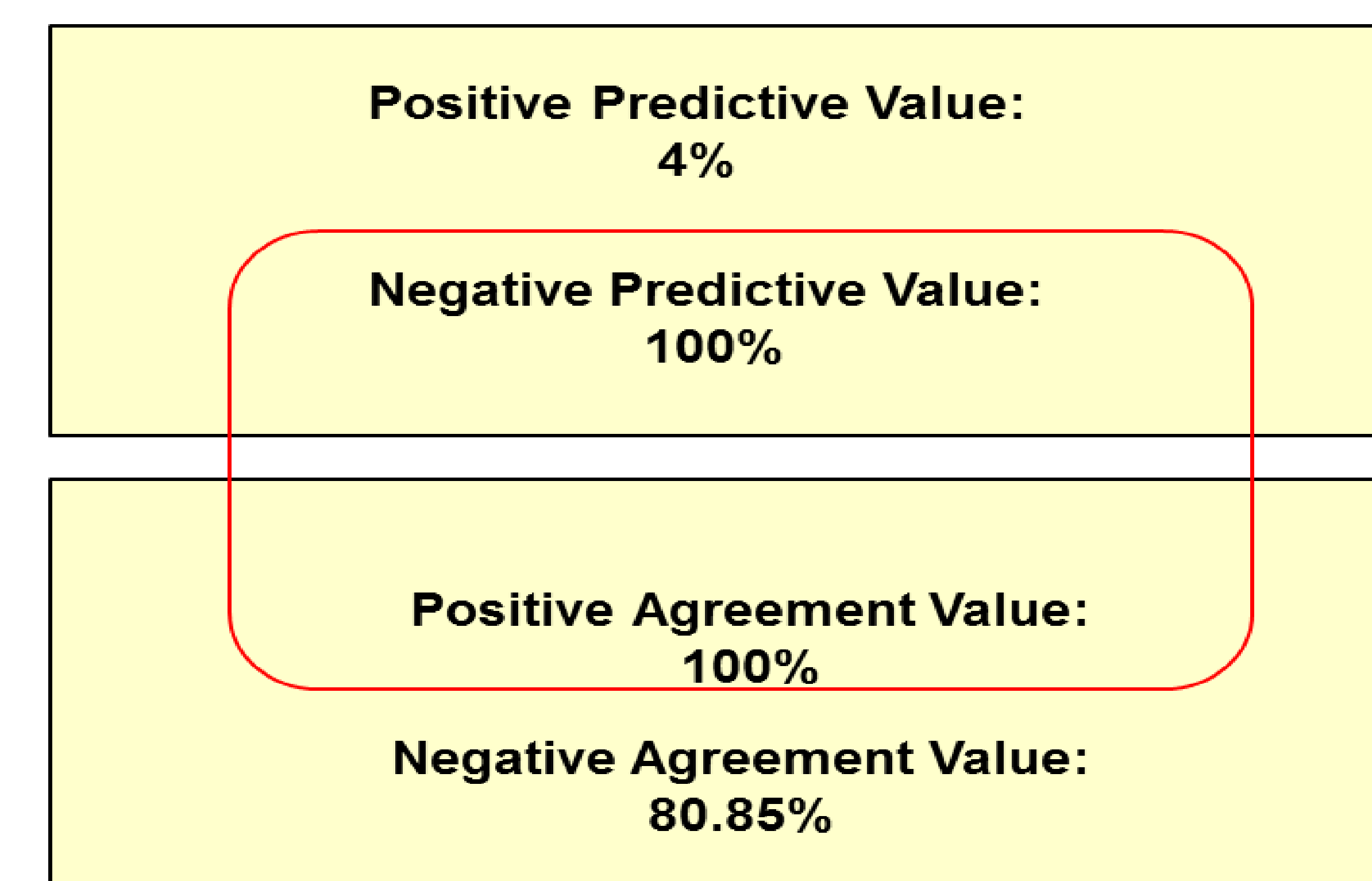
Negative Agreement Value: 80.85%

Fig. 3. Bluescreen Performance Parameters

Conclusion

The current data show:

- Bluescreen HC™ can be potentially used to screen large compound library

- Bluescreen HC™ is an ideal assay to predict non genotoxic materials with 100% accuracy

- The assay has shown more false positive which can be considered to be a more conservative approach to screen materials.