Improving throughput for assessing nephelometric turbidity units (NTUs) using the NEPHELOstar® *Plus*

Carl Peters
BMG LABTECH Inc., NC, USA

- **The NEPHELOstar *Plus* is able to detect NTU's to an LOD comparable with industry standard instrumentation**
- **A significant savings in time, effort and sample can be achieved using this approach**

Introduction

Turbidity is a fairly general concept that is used to describe the dispersion of particulate matter within a liquid phase. Measurement of turbidity is classically important in water quality testing where turbidity is often expressed in NTUs (nephelometric turbidity units). Assessing turbidity is also beneficial in other applications including biological samples where quantifying turbidity is important for measuring bacteria cell growth or antibody precipitation.
NTU values can be derived from a calibrated nephelometer or turbidimeter such as the HACH® 2100N turbidimeter; however, collecting turbidity data with a turbidimeter is a time consuming, multistep process that employs large sample volumes. Here, we provide a higher throughput, simplified, low volume approach to the collection of NTU data using the NEPHELOstar *Plus*. We present data directly comparing the NEPHELOstar *Plus*, expressed in RNUs (relative nephelometry units), to the NTU results from a HACH® turbidimeter.

Assay Principle

The nephelometry method determines the cloudiness of a solution by passing a light beam through the sample. The light is scattered by the suspended particles and the forward scattered light measured to indicate the amount of suspended particles in the solution.
The NEPHELOstar *Plus* uses a laser diode at 635 nm with adjustable diameter and intensity as a light source. Light scattered by an angle of up to 80 degree is collected using an Ulbricht sphere (Figure 1).

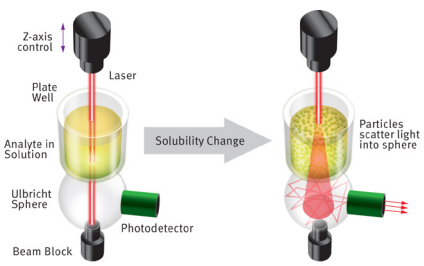

Fig. 1: NEPHELOstar *Plus* detection principle. A) Samples with little to no particles do not exhibit light scattering, light does not reach the photodetector and RNUs are low. B) Particles in solution scatter light, which reaches the photodetector and RNUs are higher.

Materials & Methods

- NEPHELOstar *Plus* microplate reader from BMG LABTECH
- UV-star 96-well clear microplates from Greiner
- HACH® 2100N Turbidimeter
- StabCal® Calibration Set 0 to 4000 NTU
- Sample cells for ratio turbidimeter
- Silicone oil
- Deionized (demineralized) water
- Stabilized formazin turbidity standards (0.063 –1000 NTU)

The turbidimeter was calibrated per instrument instructions. Blank (water) or formazin standard NTU values were recorded after the value stabilized for approximately one minute following the instrument instructions. The sample volume for turbidimeter measurements was approximately 30 ml. For experiments using the NEPHELOstar *Plus*, 200 µl of water or turbidity standards were pipetted into microplates and read using the following settings.

Instrument settings

Measurement mode:	Endpoint
Measurement interval:	1 second
Laser intensity:	80% (or as indicated)
Beam focus:	2.5 mm
Settling time:	1 second

Data analysis

For each formazin standard, eight replicate readings using the turbidimeter were averaged with Excel. These data were used to generate a standard curve. Likewise, averaged NTU data from eight replicates of the different formazin standards were used for calculating the standard curve with the MARS analysis software. LOD was calculated from at least 20 blanks using the formula:

$$LOD = Average_{blank} + (3 \times SD_{blank})$$

Results & Discussion

We first sought to determine the limit of detection or LOD for the turbidimeter using water as a blank (n = 20) and 8 replicate readings from 8 different formazin standards (0.063 to 100 NTUs). The readings in the applied range resulted in a linear standard curve with a r^2 of 0.9999. The limit of detection was calculated to be 0.119 NTUs for the Turbidimeter (Figure 2).

Rev. 06/2017
Keywords: Microbial growth, Solubility, Water quality

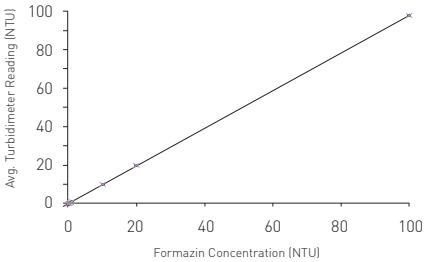

Fig. 2: Linear fit for Turbidimetric LOD. Formazin of 8 different concentrations were read using a HACH® 2100N turbidimeter and exhibit a linear fit (r^2 = 0.9999).

We then determined the NTU LOD for the microplate reader NEPHELOstar *Plus* using water as a blank (n = 32) and 8 replicates of formazin standards (NTU values ranged from 0.057 to 97.912 based on the turbidimeter results from the HACH®). Outliers were detected using Grubbs' test. Figure 3 shows that the data conforms to a linear fit.

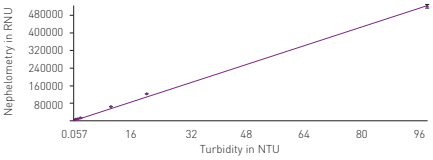

Fig. 3: Linear fit of Nephelometric vs. Turbidimetric Data Formazin of 8 different concentrations were read using a HACH® 2100N turbidimeter and subsequently using the NEPHELOstar *Plus*. The results indicate a good linear fit to over 90 NTU (r^2 = 0.999).

From these tests a comparison can be made based on performance, experimental reading time and sample volume (see Table 1). The NEPHELOstar *Plus* is competitive in terms of sensitivity and is superior in all other metrics.

Tab. 1: NEPHELOstar *Plus* vs. Turbidimeter.

	LOD (NTU)	Read-time	Sample volume
NEPHELOstar *Plus*	0.363	2 seconds per well	200 µl
Turbmidimeter	0.119	~ 1 minute per sample	~ 30 mL

Our final tests were intended to explore the range of detection of the NEPHELOstar *Plus* at higher NTUs. Once again we first assessed the average NTU values on the turbidimeter for formazin standards and compared these results to replicate readings of the same standard on the nephelometric microplate reader (figure 4).

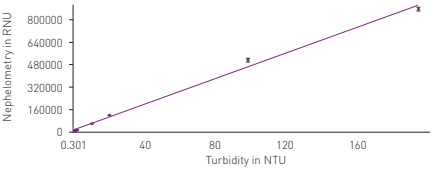

Fig. 4: Linear fit Nephelometric vs. Turbidimetric High NTUs. Formazin of 9 different concentrations were read using a HACH® 2100N turbidimeter and subsequently using the NEPHELOstar *Plus*. The two highest NTU standards (779 and 971 NTU) were eliminated due to a lack of linear correlation. However, it is of potential interest that including the higher standards in the data set resulted in a regression that correlates with a 4-parameter fit (r^2 = 0.9998) (not shown).

As figure 3 shows, a linear relationship (r^2 = 0.995) is maintained to at least the standard that measured ~194 NTUs. We also assessed whether decreasing laser intensity improved linear correlation and did not see a positive impact (data not shown).

Conclusion

The NEPHELOstar *Plus* can detect NTUs with an LOD of <0.4 NTU, while having a linear relationship between RNUs and NTUs to ~200 NTUs (r^2 =0.995). These results can be obtained with a read time of less than 2 seconds per sample, compared to over 1 minute per sample on a turbidimeter. While using 0.2 ml on the NEPHELOstar *Plus*, the turbidimeter required 30 ml per sample.