# Rapid detection of falsified or substandard vaccines using portable battery-operated TD-NMR.

Massimiliano La Colla, Jeffrey Bernstein, Wendy Coco and Nelson Stacks
WaveGuide Corporation, Waltham, MA, USA

International Society for VACCINES 2023 ISV Annual Congress
Lausanne, Switzerland

## Abstract

Worldwide, an estimated 10% of all medicines are counterfeit, resulting in approximately 300,000 deaths and costing over $200 Billion U.S. dollars annually. More recently, global counterfeiting of vaccines has become an increasing problem, posing risks to public health, loss of confidence in medicines, healthcare providers, and health systems. More than ever, it is of extreme importance to monitor and guarantee vaccine authenticity through the supply chain down to the patients. Chromatography and Spectroscopy techniques are the most effective methods to guarantee the quality and safety of vaccines and other pharmaceutical products. However, most of these analytical techniques are for in-laboratory use, require large amounts of vaccine for testing, are expensive, time consuming, and necessitate skilled personnel to operate. Currently, there are no field methods able to check vaccine authentication quickly and reliably. In this context of traceability, WaveGuide is developing a portable µNMR instrument which has shown the ability to distinguish authentic from substandard or falsified vaccines. The WaveGuide instrument utilizes time-domain NMR to measure the relaxation time characteristics ($T_2$) of the authentic biological sample. The relaxation time characteristics are unique to the bulk sample chemistry, and physical properties, such as concentration, conformation, and viscosity. The measured $T_2$ relaxation decays are fitted with both single- and bi-exponential decay models, and the corresponding decay-times and component amplitudes are determined and analyzed. This novel time-domain NMR technique can distinguish authentic vaccines from surrogate solutions, diluted originals, verified vaccines from various manufacturers, and in some cases distinguish across different storage conditions. Such analysis can be exploited for other types of biopharma assets as well as consumer products for authentication, forensics, and supply chain integrity. WaveGuide's portable µNMR is the first battery-powered instrument that is highly automated and robust enough to operate in the field without the need for a trained NMR technician.


The WaveGuide Formµla™ portable µNMR

## Introduction

According to the World Health Organization (WHO), the consumption of counterfeit and substandard medicines is a global problem, especially in developing countries where 25% to 50% of the population consume adulterated drugs[1].

Vaccines are extremely important to improve human health worldwide and are one of the most effective ways to control and contain the transmission of infectious diseases and prevent some types of cancer[2].

The total number of "discovery incidents" — discrete events triggered by the discovery of counterfeit, illegally diverted, or stolen materials — more than doubled between 2014 and 2020. The high demand for efficacious vaccines associated with the unfortunate distribution of falsified or substandard vaccines has become a global issue. An example is the recent COVID-19 crisis, characterized by a shortage of vaccines, a high global demand with an unbalanced distribution which fueled the production of falsified or substandard vaccines around the world[3,4,5].

Chromatography and Spectroscopy techniques are the most effective methods to guarantee the quality and safety of vaccines and other pharmaceutical products. However, most of these analytical techniques are for in-laboratory use, require large amounts of vaccine for testing, are expensive, time consuming, and need skilled personnel.

WaveGuide Corporation has a portable µNMR instrument which has shown the ability to distinguish authentic vaccines from falsified vaccine surrogates[6]. The WaveGuide instrument utilizes TD-NMR to measure the relaxation time characteristics (T2) of the authentic biological sample. The relaxation time characteristics are unique to the bulk sample chemistry, and physical properties, such as concentration, conformation, and viscosity of the sample. The measurement of the T2 relaxation profile was used for these experiments because it is a rapid test that can be quite sensitive to differences in the bulk composition of a sample.

This novel time-domain NMR technique can distinguish authentic vaccines from surrogate solutions, diluted originals, and verified vaccines from various manufactures, when trained with known samples of the authentic vaccines. Such analysis can be exploited for other types of biopharma assets as well as consumer products for authentication, forensics, and supply chain integrity.

## Materials

The vaccines (Table 1) were kindly provided by the Vaccine Identity Evaluation (VIE) Consortium — University of Oxford & STFC Rutherford-Appleton Laboratory, UK.

Bexsero, Engerix, and Flucelvax were analyzed directly from the original and sealed syringes. Nimenrix, supplied as a lyophilized white powder, was reconstituted with saline in the original vial and aliquoted into glass vials prior to use.

| *Vaccine* | *Application* | *Lot #* | *Syringe/Vial #* |
|---|---|---|---|
| Bexsero | Meningitis B | ABXB90DA | 9 |
| Engerix B | Hepatitis (B) | AHBVC999AL | 3 |
| | | AHBVC986AB | 2 |
| | | AHBVC999AB | 5 |
| | | AHBVD044AF | 3 |
| Flucelvax | Influenza | 3079381A | 8 |
| | | 3079661A | 4 |
| Nimenrix | Meningitis | FE7696 | 6 |

Table 1: Vaccines samples as provided by the VIE consortium.

This study included analysis of the authentic vaccines, dilutions of the authentic vaccines with water for injection, and comparison of the authentic vaccines with a list of potential counterfeit surrogate solutions suggested by the VIE Consortium (Table 2).

| *Potential Surrogate* | *Composition* | *Source* |
|---|---|---|
| Water for Injection | - | Hospira; Lot No. FY7571 |
| Tap Water | - | Tap water from Newton, MA |
| Deionized Water | - | VWR; Cat No. 470300-966 |
| 1X PBS | Phosphate Buffer Saline | VWR; Cat No. AAJ61196-AP |
| Sucrose | 5% sucrose in water for injection | Sigma; Cat No. S7903-250G |
| Glucose | 5% glucose in water per injection | VWR, Cat No. 97061-168 |
| Mannitol | Mannitol, powder, USP. Diluted at 5% in water per injection | VWR; Cat No. 95034-334 |
| Sodium Chloride | 0.9% sodium chloride in deionized water | VWR; Cat No. BDH7257-2 |
| Gentamicin | Gentamicin Sulfate, tested at 40 mg/ml | VWR; Cat No. 10128-216 |
| Amikacin | Amikacin Sulfate, prepared and tested at 250 mg/mL in water for injection | VWR; Cat No. 103626-014 |
| Hyaluronic Acid | Juvederm, Vollurex XC, 17.5 mg/mL of Hyaluronic Acid in phosphate buffer containing 0.3% w/w lidocaine | Online store; Lot No. V17LA80744 |

Table 2: List of solutions often used as counterfeit/surrogates for authentic vaccines.

## Methods

Samples were placed in a glass tube configured for the instrument with a 3 mm outer diameter and 2.5 mm inner diameter.

The volume of sample used for each analysis was 15 µL.

The vaccines were tested straight and diluted with water for injection.

All experiments were conducted at a controlled temperature of 25˚C to minimize the difference in temperature-dependencies on the relaxation time.

Five to twenty replicates were measured for each sample.

## Measurements and Analysis

A standard CPMG (Carr-Purcell-Meiboom-Gill) acquisition pulse sequence was utilized to measure the T2 relaxation[7,8] profile of the vaccines.

For the results reported here, the measured T2 relaxation decays were fitted with both single- and bi-exponential decay models, and the corresponding decay-times and component amplitudes were determined and analyzed.

## References

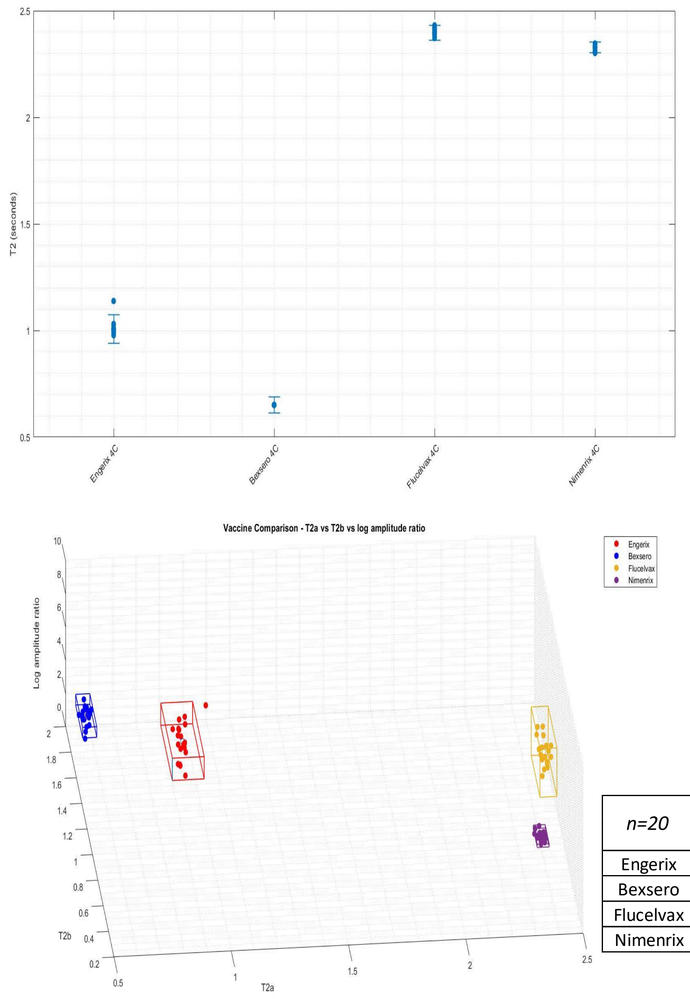
1. WHO (World Health Organization, Geneva, Switzerland); 2006. Counterfeit Medicines, Fact Sheet 275, (revised Feb. 2006).
2. Rodrigues CMC, Plotkin SA. Impact of Vaccines; Health, Economic and Social Perspectives. Front Microbiol. 2020 Jul 14; 11:1526. doi: 10.3389/fmicb.2020.01526. PMID: 32760367; PMCID: PMC7371956.
3. https://www.rovi.es/en/content/recall-notification-lot-000190a(lot #000190A) of the COVID-19 vaccine (Spikevax® recalled: 764,900 doses.
4. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/north-coast-biologics-607532-05212020
5. https://www.iddo.org/document/medical-product-quality-report-covid-19-issues-issue-15-september-2022-data-january-march
6. https://www.bloomberg.com/press-releases/2020-02-25/waveguide-launches-world-s-first-portable-nmr-device-the-waveguide-form-la
7. H.Y. Carr, E. M. Purcell, "Effects of Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments", *Phys. Rev.*, 94, 630 (1954).
8. S.Meiboom, D. Gill, 1958. "Modified Spin-Echo Method for Measuring Nuclear Relaxation Times", *Rev. Sci. Instrum.*, 29, 688 (1958).

## Disclosures

WaveGuide Corporation is a member of the Vaccine Identity Evaluation Consortium with the University of Oxford & STFC Rutherford Appleton Laboratory, UK

## Results

Profiling of an authentic vaccine: For each vaccine tested, we established a reliable metric that provides little variation over different lots, syringes/vials, and determined that this metric can accurately distinguish each of the four vaccines.



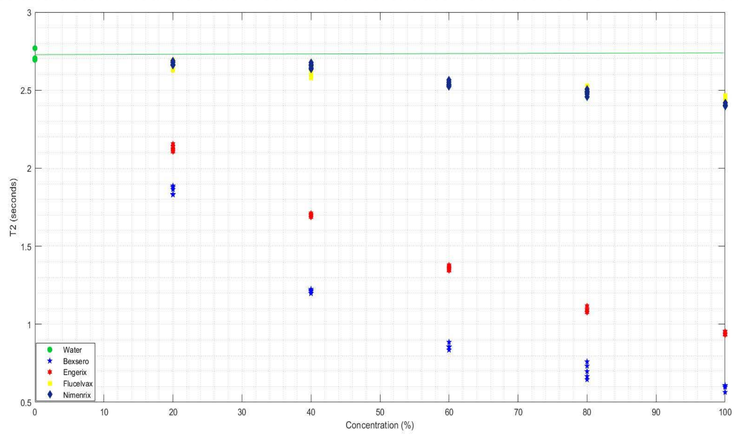

- Analysis with single-component exponential model
- The four vaccines are distinguishable from each other based on the single-component T2 value
- Bexsero and Engerix B are most easily distinguishable
- Flucelvax and Nimenrix have a similar single-component T2 value but are easily distinguished from Bexsero and Engerix

| *n=20* | *Single exponential T2* | |
|---|---|---|
| | *Avg.* | *St. Dev* |
| Engerix | 1.007 | 0.034 |
| Bexsero | 0.651 | 0.019 |
| Flucelvax | 2.397 | 0.018 |
| Nimenrix | 2.328 | 0.012 |

- Analysis with two component bi-exponential model
- Bexsero, Engerix B, Flucelvax, and Nimenrix are easily distinguishable based on the bi-exponential analysis

| *n=20* | *Bi-exponential T2a* | | *Bi-exponential T2b* | | *Log Amplitude Ratio* | |
|---|---|---|---|---|---|---|
| | *Avg.* | *St. Dev.* | *Avg.* | *St. Dev.* | *Avg.* | *St. Dev.* |
| Engerix | 0.949 | 0.034 | 1.563 | 0.108 | 2.174 | 0.334 |
| Bexsero | 0.564 | 0.015 | 1.786 | 0.065 | 2.246 | 0.159 |
| Flucelvax | 2.400 | 0.018 | 0.779 | 0.081 | 6.523 | 0.729 |
| Nimenrix | 2.351 | 0.013 | 0.462 | 0.037 | 3.933 | 0.051 |

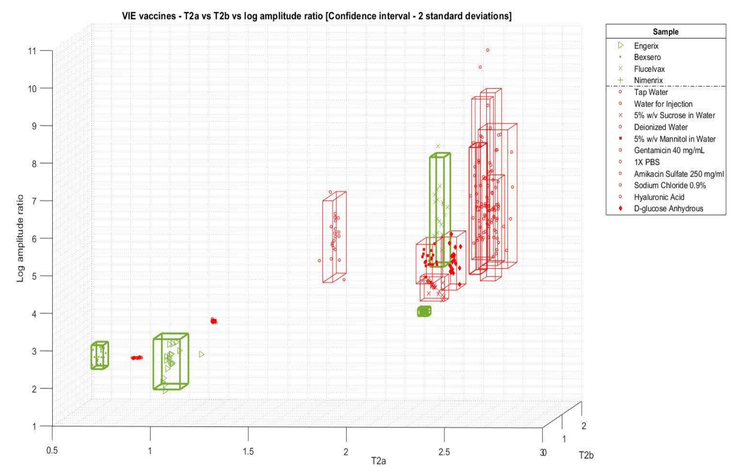
Dilution of authentic vaccine: The metric established in the experiments above were compared with various dilutions of the authentic vaccines and determined that this metric can differentiate neat vaccines from their dilution.




- Analysis with single-component exponential model
- For Bexsero and Engerix, the T2 value changes significantly as a function of the vaccines concentration when diluted with water
- For Flucelvax and Nimenrix, the T2 value changes slightly and roughly linearly as a function of the vaccines concentration when diluted with water

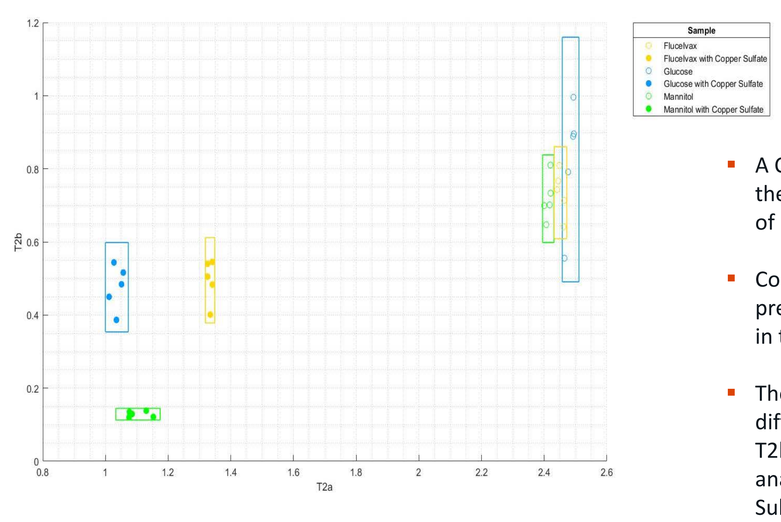
| *n=5* | 0 | | 20% | | 40% | | 60% | | 80% | | 100% | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Avg.* | *St. Dev.* | *Avg.* | *St. Dev.* | *Avg.* | *St. Dev.* | *Avg.* | *St. Dev.* | *Avg.* | *St. Dev.* | *Avg.* | *St. Dev.* |
| Engerix | - | - | 2.127 | 0.019 | 1.698 | 0.009 | 1.360 | 0.014 | 1.092 | 0.015 | 0.942 | 0.009 |
| Bexsero | - | - | 1.859 | 0.026 | 1.210 | 0.011 | 0.854 | 0.020 | 0.700 | 0.047 | 0.595 | 0.018 |
| Flucelvax | - | - | 2.671 | 0.024 | 2.601 | 0.019 | 2.545 | 0.012 | 2.494 | 0.018 | 2.442 | 0.012 |
| Nimenrix | - | - | 2.669 | 0.012 | 2.654 | 0.017 | 2.545 | 0.018 | 2.483 | 0.019 | 2.405 | 0.007 |
| Water | 2.715 | 0.031 | - | - | - | - | - | - | - | - | - | - |

Detection of falsified or substandard vaccine: The four authentic vaccines were compared with several surrogate solutions. The four authentic vaccines are colored in green, the surrogate solutions are colored in red.




- Bexsero, Engerix and Nimenrix are clearly distinguishable from all surrogate solutions
- Flucelvax is easily distinguishable from most surrogate solution, but not distinguishable from the mannitol and D-glucose solutions

Flucelvax vs Mannitol/Glucose: WaveGuide Corporation developed an alternative method to better differentiate Flucelvax from Mannitol and Glucose using a solution of Copper Sulfate.




- A Copper Sulfate solutions was spiked into the Flucelvax vaccine and into the solutions of mannitol and glucose
- Copper Sulfate binds to the hemagglutinin present in the Flucelvax vaccine but absent in the Mannitol and Glucose solutions
- The addition of Copper Sulfate resulted in a different bi-exponential profile (T2a and T2b) which was better separated than the analysis of these samples without Copper Sulfate

## Conclusions

- The Waveguide TD-NMR instrument reliably detects and differentiates authentic vaccines
- The Waveguide TD-NMR instrument enables high accuracy in differentiating authentic from diluted and/or inauthentic products
- The Waveguide TD-NMR instrument could be a complementary and/or alternative method for vaccines authentication