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Facile biosensor-based system for on-site quantification of total viable counts in food and environmental swabs

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ABSTRACT

We describe a new biosensor platform for rapid and simple quantification of total aerobic viable counts of bacteria (TVC) in food and environmental swabs by oxygen respirometry. The system uses disposable swab vials with phosphorescent oxygen sensors integrated in the bottom part, a small block heater/incubator and a handheld sensor reader. In the testing, groups of 1–20 swabs samples were prepared using the standard method (ISO, 18593:2018) in sensor vials, which were then incubated at 30 °C and measured hourly in a contactless, non-invasive manner. The measurements reveal time profiles of dissolved O_2 in each sample vial, from which Threshold Time of sensor signal was determined and then TVC values (CFU/cm^2) were calculated using the calibration equation. The method covers the range of 0.65–7.87 Log (CFU/cm^2) and produces results in 1–8 hrs. The test was validated with swab samples from surfaces contaminated with *E. coli,* with whole meat microbiota, and with real environmental swabs. The results showed no statistically significant difference with the reference method which takes 48–72 h. The swab testing platform is fast and accurate, simple (sample-and-measure), portable, low cost (<$5k), requires no serial dilutions and is suitable for on-site deployment and use.

1. Introduction

Microbial contamination is of major concern for many industrial sectors, particularly the food industry. It can occur from contaminated equipment surfaces, insufficient hygiene, food processing personnel, and from food products themselves (Jones et al., 2020). Although food processing plants have strict guidelines to minimise microbial contamination, reservoirs of possible pathogens can occur within various equipment and parts of the food processing chains. It is estimated that 10% of the population is affected by food-borne pathogens which in turn have led to 420 000 deaths in 2010 globally (Elshahat et al., 2019).

Microbial swab testing is a fundamental component of surface sampling and process monitoring, especially in the food, pharmaceutical and biotech industries (Laranjo et al., 2019; Sandle, 2016). Environmental and microbiological surveillance is currently conducted through the routine surface sampling of food contact surfaces using a variety of swabs: brush swabs, sponge swabs or integrated swabbing devices (Fig. 1). Swab testing, aimed at tracing and controlling baseline levels of contamination and cross-contamination, is a key point in environmental, hygiene and safety monitoring (Jones et al., 2020). Swabbing is conducted routinely and periodically at multiple points of each production facility and process, as part of microbiological surveillance, safety, and quality assurance. The swabs are then analysed using microbiological culture-dependent methods, molecular methods (PCR, ELISA) or instrumental techniques such as flow cytometry, microscopy, mass spectrometry, and microcalorimetry (Alamer et al., 2018; Braissant et al., 2010; Kasturi and Drgon, 2017; Ou et al., 2019).

Among these readout methods, determination of total viable counts of aerobic bacteria (TVC, CFU/cm^2) by culturing them on non-selective media (ISO, 18593:2018) represents the largest and still indispensable swab testing group. However, this method is labour intensive and time-consuming. Although many improvements in the traditional workflows have been introduced, the current techniques still require laboratory settings and some experience from the user, are slow (results are achieved after 24–72 h) and can be easily overloaded with samples, producing inconclusive results (Hauge et al., 2017). Moreover,

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conventional swab testing for TVC usually involves several steps clearly separated in time and space (Fig. 2A), which makes automation and upscaling difficult. External or centralised labs are often used for swab analysis to which collected swabs are transported on ice over long distances. This not only further delays the already lengthy time to result, but also exposes swab samples to additional stress and manipulation. As a result, the industry is constantly looking for advanced methods that are quick (same day or even same shift result), reliable, automated and affordable for routine microbiological surveillance.

Optical oxygen respirometry offers an automated, user-friendly and quick alternative to conventional TVC testing (O'Mahony et al., 2005; Zitova et al., 2009). This detection method relies on dedicated sensor materials (solid-state coatings or soluble probes) introduced to the samples, phosphorescence of which is quenched by sample dissolved O_2, resulting in low phosphorescent signals corresponding to high O_2 levels and vice versa (Papkovsky and Dmitriev, 2013). Oxygen respirometry has a wide ranging applications which includes the analysis of mammalian cells, isolated mitochondria and small animal metabolism (Hynes et al., 2006; Will et al., 2007), the enumeration of bacteria in food samples (Hempel et al., 2011), and the effects of antimicrobials (Elisseeva et al., 2020). Thus far, this methodology has not been adapted for handling swab samples and the analysis of their TVCs.

In this paper, we describe a simple and transportable system for swab testing, which uses disposable swab vials with built in phosphorescent oxygen sensors and in which groups of swab samples are analysed via contactless, real-time monitoring of their microbial growth via oxygen respiration, to give rapid and quantitative readout of their TVC values (CFU/cm^2) (Fig. 2B). The system is integrated with existing swab vials (having a cotton tip and medium, sterile) and/or bio-containers, a portable block heater/incubator, an autonomous handheld sensor reader FirestingGO2 (Pyroscience GmbH). It is configured as a simple sample-and-measure platform, which can be deployed at various sites and operated by non-skilled personnel.

2. Materials and methods

2.1. Materials

Plate count agar (PCA) was purchased from Sigma-Aldrich Merck (Dublin, Ireland) while Nutrient Broth (NB) and Maximum Recovery Diluent (MRD) were purchased from Oxoid (Dublin, Ireland). Sterile 10 mL swab tubes were from Sarstedt (Germany). Stock cultures of *Escherichia coli* NCIMB 11943 *(E. coli)* were obtained from the School of Microbiology, University College Cork and stored at −80 °C in 80% glycerol in LB broth. Working cultures were prepared by inoculating 50 μL of semi-defrosted stock into 5 mL of LB broth and incubating at 37 °C on a rotary shaker at 250 rpm until an OD600nm of ≅ 0.8 was reached (typically overnight).

The polymeric cocktail for making the phosphorescent oxygen sensitive coatings, which comprised polymeric microparticles impregnated with a Pt (II)-tetrabenzoporphyrin (PtBP) dye and suspended at 40 mg/mL concentration in 95% ethanol also containing 5% hydrogel as polymeric binder, was kindly provided by Agilent Ireland (Cork). This sensor cocktail (stored at 4 °C in the dark) was sonicated for 2 min (Fisherbrand Ultrasonic Bath FB15047, Fisher Scientific, United Kingdom) and quickly vortexed. 5 μL of the sensor cocktail was dispensed onto the bottom side wall of sterile vials in a sterile environment, using a Distriman repetitive pipette. All tubes were left to air dry in a laminar flow hood for 1 h, then sealed with caps to maintain sterility. Typically, batches of 50–100 sensor tubes were produced and then stored in a dark place at room temperature for up to 3 months until further use.

2.2. Measurement of sensor signals in the tubes

The autonomous hand-held sensor reader FirestingGO_2 was used to measure the phosphorescence intensity (mV units) and phase shift (degrees angle units, dphi) signals from the sensor. Batches of up to 20 sensor tubes were incubated at 30 °C for 10 h and the sensor signal, dphi, was measured manually every hour through the sensor located on the side of the vial. Care was taken as to ensure no mixing of the vial contents occurred when measurements were taken.

O_2 calibration of sensor vials was produced by placing the 10 mL testing vial with NB in a water bath (equilibrated at 30 °C) and purging through it sequentially with standard O_2/N2 gas mixtures produced by a precision gas mixer/tonometer LNI (Switzerland) and gradually changing O_2 content from 21 kPa (21%) to 0 kPa. After temperature and O_2 equilibration of the vial (seen as stable phase readings), steady state dphi signals were recorded with the FirestingGO_2 and then plotted as a function of O_2 concentration in Excel. An analytical equation relating O_2 concentration to dphi was obtained as described in section *3.4*.

2.3. Reference culture-based TVC method

Conventional plate count method (ISO, 18593, 2018) for total aerobic viable counts (CFU/cm^2) by agar plating was used for comparison. The aforementioned samples (artificially contaminated with *E. coli* or natural meat microbiota, and environmental swabs) were plated on PCA plates in duplicate, incubated for 48–72 h at 30 °C. Grown colonies were enumerated using an automatic counter pen (VWR International, Dublin, Ireland) and initial microbial load (CFU/cm^2) was calculated accordingly.

2.4. System setup, optimisation, and calibration for swab testing

To establish the relationship between the primary signal, dphi, and initial bacterial load (Log (CFU/mL) or Log (CFU/cm^2)), the sensor-based swab testing system was calibrated using *E. coli*. Serial dilutions (1 in 10) of overnight *E. coli* culture (approximately 10^9 CFU/mL) were prepared using 9 mL MRD for a concentration range of 10^8–10^3 CFU/mL per tube. For the 10 mL sensor vials, 1 mL of a given dilution (at known concentration) of *E. coli* was added to 9 mL of NB. The vials were incubated in a portable incubator at 30 °C and sensor signal was measured hourly for 10 h using the FirestingGO_2. Parallel plate counting

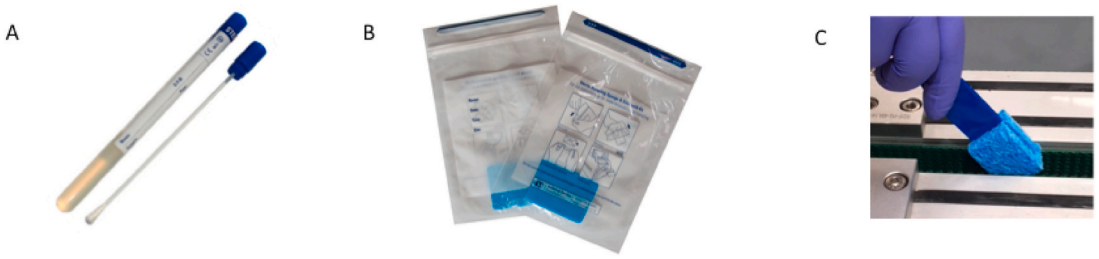

Fig. 1. Different swab types commercially available for environmental and hygiene swabbing. A: Cotton tip swab; B: Carcass sampling sponge; C: EnviroStik (sponge with breakpoint handle).

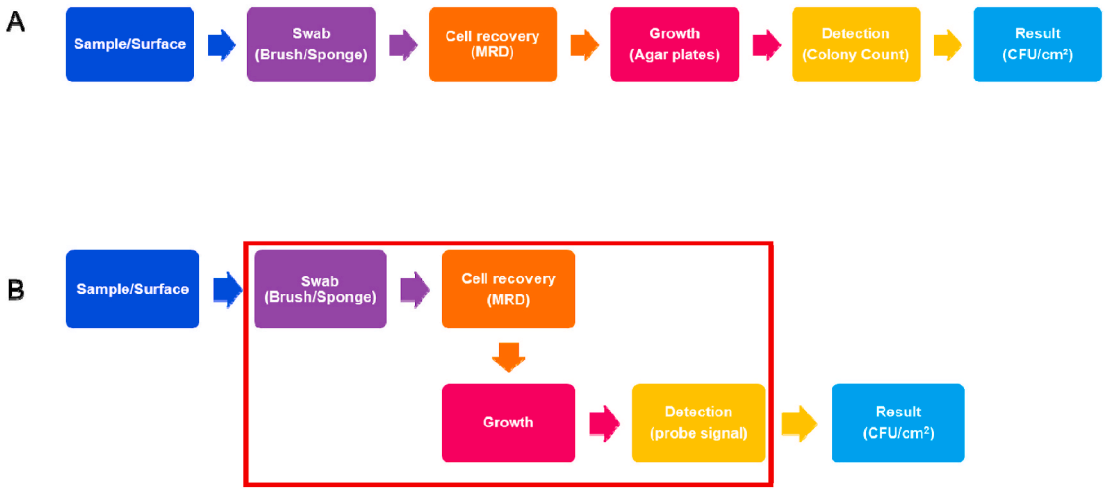

Fig. 2. Workflow for microbial swab TVC testing. A: traditional method, B: sensor based method. The proposed system retains the original sample preparation procedure but allows for the integration of the key functions (red rectangle) into one disposable element – sensor vial.

was performed as described in 2.3.

2.5. Preparation and analysis of swab samples

Validation of the testing system was done by analysing surfaces: i) artificially contaminated with *E. coli*, ii) contaminated with natural meat microbiota, using 10 × 10 cm plastic trays with lids (Sarstedt, Germany), and iii) environmental swabs. In the first case, an overnight *E. coli* culture was serially diluted 1:10 in 20 mL of MRD for concentrations ranging 10^8 to 10^4 CFU/cm^2. These diluted solutions of *E. coli* were transferred into individual 30 mL plastic spray bottles (bought from a local chemist, sterilised with 70% ethanol), from which two pumps of the spray (≈200 μL) were applied on each surface of the plastic tray and allowed to dry in air for 30 min. Contamination of surfaces was done in replicate as to use swabs for both the sensor system and the reference method (ISO, 18593:2018). Swabs from the 10 mL vials (with sensor coating and without), pre-wetted with approximately 1 mL MRD, were used to swab each surface as per standard procedure. For the sensor system, 10 mL of NB was added directly to the vial and initial readings were taken using the FirestingGO_2 before incubation.

In the second case, meat samples (n = 9) purchased from a local butcher were placed individually into 10 × 10cm plastic trays, such that their bottom side was covering the tray surface, while the top surface was in contact with air (aerobic environment). The samples were stored in the cold room at 4 ± 1 °C for 24 h, 48 h or 72 h. After this, each meat sample was flipped into the top part of the tray to create an imprint of the top side and ensure proper transfer of microbiota from the surface of the meat. Pre-wetted (MRD) swabs from the 10 mL sensor vials were used to swab the tray surfaces with meat sample imprints, using a different swab for the bottom and top of the tray. 10 mL of NB was added to each sensor vial and vortexed for approximately 30s. 200 μL of the resulting suspension was taken from the vial for analysis via agar plating method (ISO, 18593:2018), while the remaining solution in the sensor vial was incubated at 30 °C, with hourly measurements of sensor signals. Initial readings of sensor vials were also taken before incubation.

For the third case, samples were obtained by swabbing (according to ISO, 18593:2018) laboratory surfaces such as sinks, benchtops, etc. With pre-wetted (MRD) swabs from the 10 mL vials. Swabbing of surfaces was done in replicate as to analyse the samples using the sensor system and the reference method described in 2.3. Environmental swabs were analysed exactly as the artificial contamination swabs were (see above).

2.6. Data processing and statistics

The dphi values recorded for the vials with FirestingGO_2 reader were extracted from the instrument using Pure Oxygen Logger software (Pyro-Science) and underwent statistical analysis using SigmaPlot 11 (Systat Software Inc., USA). The consistency of test vials was analysed using the Mann Whitney Wilcoxon Test, comparing phase and intensity signals obtained in dry vials and in vials containing NB. In respiration tests, time profiles of the phase signal for each assay vial were plotted, then sigmoidal fitting using the Dynamic Fitting Tool in SigmaPlot was applied and signal threshold time, TT (h), was determined for each sample. By applying the calibration equation obtained with pure *E. coli* culture, these TT values were converted into CFU/mL values and, by applying an additional 0.1x conversion factor into CFU/cm^2 values, which reflect the bacterial load on a 100 cm^2 surface. In addition, non-linear regression analysis of the phase signal data with the four-parameter sigmoidal model was performed (Santovito et al., 2019). One-way ANOVA test (P = 0.05) and Pearson correlation analysis were done to compare results obtained with the sensor method and reference method. Blant-Altman analysis with Shapiro-Wilk normality test was used to compare the sensor based method with the reference method for total viable counts on swabbed surfaces (Tanaka et al., 2010). Unless otherwise stated, all experiments were conducted in triplicate on different days.

3. Results and discussion

3.1. Platform design for swab testing

Previous respirometric platforms were realised as stationary lab-based systems, with rather sophisticated, bulky and expensive detectors - benchtop plate readers or carousel vial readers (Fernandes et al., 2013; Hempel et al., 2011; Hynes et al., 2006; O'Mahony and Papkovsky, 2006). Typical start-up costs for such platforms are in the range of $25-35 k, which is prohibitively high for potential small users, particularly in the food industry. Also these platforms do not fully utilise the recent advancements in O_2 sensing instrumentation and sensor materials which have enabled the creation of compact, flexible, low-cost, user-friendly and autonomous bioanalytical platforms.

Based on this knowledge and the available commercial components, we have designed a new sensor-based system dedicated for facile, rapid and distributed testing of swab samples. The system is simple, modular and flexible and its main components include: i) disposable testing vials, each containing a built-in swabbing tool and an O_2 sensor coatings (such

vials were produced in-house in several modifications); ii) an autonomous handheld reader with data logger, FirestingGO_2, which can read sensor signals from the vials in a non-invasive manner; iii) a block heater in which the vials are maintained at optimal temperature during the assay, normally at 30 °C. One reader and block heater usually serve a panel of 5–15 assay vials, which can be analysed simultaneously or asynchronously. Standard gas incubator with a pebble tray, a water bath or even an egg incubator can also be used for incubating tested samples at 30 °C. Images of the system components and experimental settings for swab testing are shown in Fig. 3.

The key component of the whole system is the sensor vial, designed based on the existing swabbing or bioassay vials. Swabbing in environmental and process control applications is well established, with several commercial devices dedicated for swab sampling are available (see Fig. 1A) and used routinely by numerous labs according to standard protocols. By incorporating an O_2 sensor coatings in such swabbing vials, we have retained their original sample preparation function, while also enabling the subsequent respirometric analysis of the swabbed material within the vial. The latter is performed by simply incubating the vials on a block heater and measuring their sensor signals periodically and in a contactless manner with an external autonomous reader FirestingGO_2. Thus, a range of key functions (Fig. 2B, red rectangle) are now integrated in one simple disposable element. At the same time, swab/sample preparation procedure remains unchanged and in accordance with industry standards.

3.2. Swab TVC method development

In oxygen respirometry, samples are usually sealed to reduce O_2 intake from air, which otherwise would counteract the slow bioprocess of O_2 consumption. Anaerobic cuvettes (Papkovsky et al., 2009) or oil seal applied on top of the samples measured in microtiter plates (Elisseeva et al., 2020; Jasionek et al., 2013; O'Mahony and Papkovsky, 2006) are often used. However, for relatively large samples containing rapidly growing bacterial cells, sealing appears to be unnecessary. The O_2 barrier properties of the liquid medium are sufficient, especially when the sensor coating is at the bottom of the vial and O_2 is measured locally. Thus, vials with crude liquid samples and air headspace (also capped to prevent microbial contamination or spillage) can be handled and analysed routinely by respirometry.

Cell respiration and growth in a confined liquid sample causes a transition from initially aerated condition (high, atmospheric O_2) to low O_2 state. Respirometry of mammalian cells normally deals with slow and gradual deoxygenation profiles, for which the slopes reflecting sample oxygen consumption rate (OCR, i.e., d [O_2]/dt) are usually measured. In contrast, respiration of bacterial cultures produces sigmoidal profiles in which transition from high to low O_2 is steep and occurs within a narrow time frame, after a critical cell density is reached (Jasionek et al., 2013). This feature is due to the fast growth of bacteria and exponential increase in cell numbers during the assay. It allows for accurate enumeration of bacterial cells in the original sample, based on Threshold Time (TT, hours) of the optical signal or O_2 concentration determined from the respiration profile.

The highly automated and integrated systems, such as plate or carousel-based readers, can take sensor readings every 5–15 min and produce smooth respiration profiles (Jasionek et al., 2013). For the manual system shown in Fig. 3, frequent reads can disturb sample/assay temperature (30 °C). Therefore, samples are usually measured every 60 min, in groups of 5–15 vials. This mode is convenient (one scan takes <1 min per batch), but produces less data points, especially in the oxygenation transition zone. For more accurate TVC quantification in swab samples, mathematical (sigmoidal) fitting of the results is desirable (though not necessary). Furthermore, an endpoint read can also be used for semi-quantitative analysis of TVC levels in swabs: below or above the set threshold, or negative/positive.

Below we describe the optimisation and practical demonstration of this system. First, with pure cultures of a model bacteria (*E. coli*) and then with real swabs produced in house according to ISO 18593:2018 protocol.

3.3. Optimisation and quality assessment of sensor vials

Initial coating of swab vials underwent optimisation due to the presence of thick plastic at the bottom of each tube which affected optical measurement. Swab vials were prepared where the sensor cocktail was dispensed in different locations while maintaining the tubes at different angles. The side of the vial was the preferable sensor location, as it gave high intensity signals and relatively easy access to the FirestingGO_2 for measurement. However, the angle at which the vials were dried was problematic. It is crucial that the sensor spot dries properly and retains its shape as this severely affects signal intensity. A 45° was not suitable for side application as the sensor would dry in a streak rather than a localised spot (data not shown). The optimal set up for swab vial preparation involved maintaining the tubes horizontal so that the sensor could be dispensed and dried in a spot on the side (Fig. 3).

Once optimal set up for sensor vial preparation was established, a larger batch of swab tubes were prepared (n = 102) and tested with the FirestingGO_2 by measuring their intensity and phase signals in air and with added 10 mL of NB (Fig. 4).

Quality assessment of sensor vials (n = 102) was done by measuring both intensity (mV) and dphi signals with FirestingGO_2. Sensors were measured first in ambient air followed by in 10 mL of Nutrient Broth (NB) at room temperature (Fig. 4A). As described in Table 1, measurements obtained in NB showed significantly different ($P \leq 0.001$, Mann

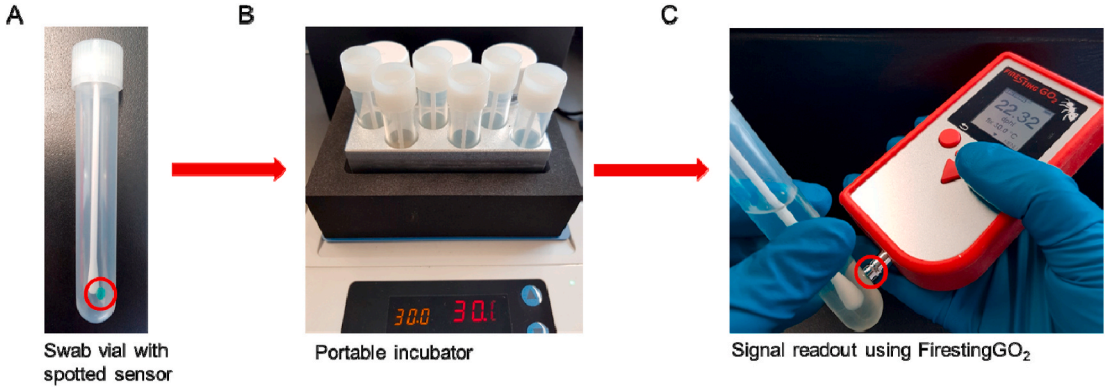

Fig. 3. The main components of the sensor-based swab testing platform. A: Disposable swab testing vial integrated with the swabbing tool (cotton tip brush) and the O_2 sensor coating (circled). B: benchtop block heater for incubating test vials at 30 °C during the assay. C: FirestingGO_2 reader with data logger scanning sensor signal from the vial.

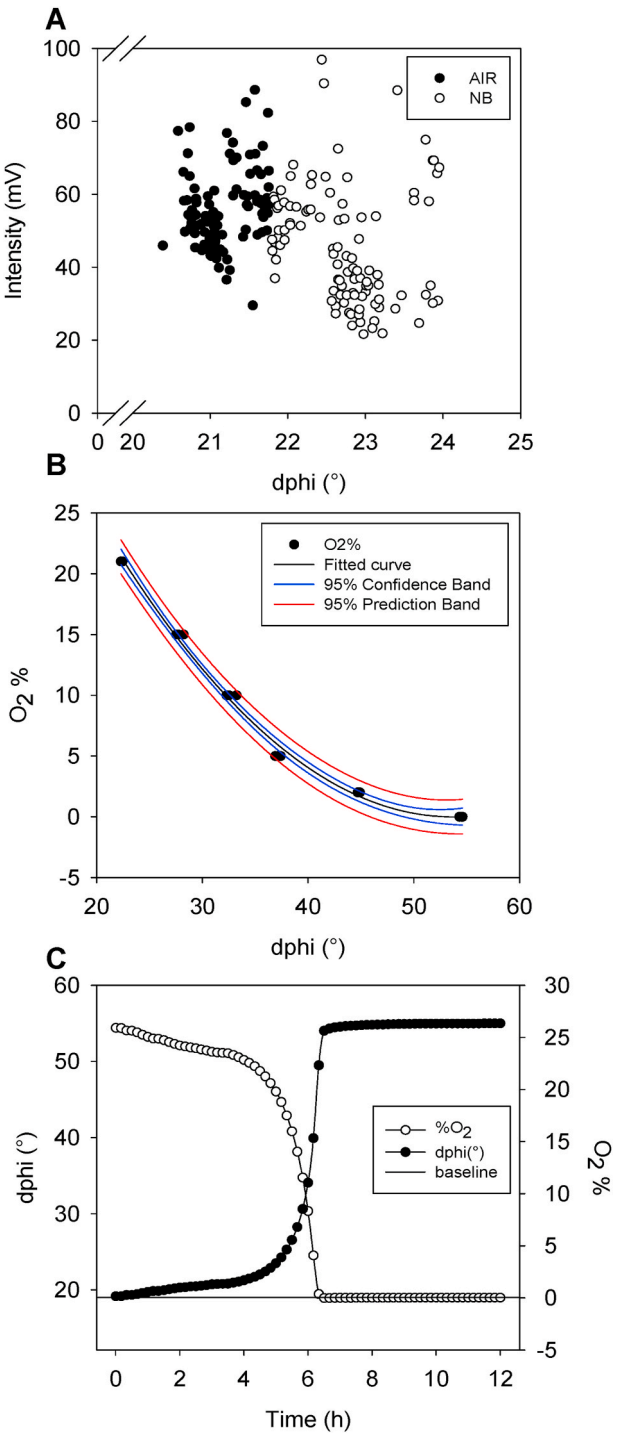

Fig. 4. Quality assessment of sensor swab vials and oxygen calibration. A: The variation of Intensity (mV) and phase signals, dphi, in dry sensor vials (AIR) and in the presence of 10 mL of nutrient broth (NB). B: Oxygen calibration curve obtained using different O_2 standards. C: oxygen respiration of *E. coli* incubated at 30 °C in sensor swab vials and measured continuously with FirestingGO$_2$ reader. Comparison of phase signal and oxygen concentration profile, the latter was calculated using the calibration equation achieved in B. Baseline signals and Time to Threshold (TT) are shown.

Whitney Wilcoxon Test) intensity and phase signals than in dry vials (AIR), with phase signal median value higher in NB than in AIR, with a difference of 1.67°. The lowest dphi signal value measured in AIR was 20.39°, and the highest measured in NB was 23.95°. Intensity signals, which are influenced by spot shape, measurement distance and

Table 1
Statistical analysis of intensity (mV) and dphi signals in dry sensor tubes (AIR) and in the presence of 10 mL of nutrient broth (NB).

Group	Mean	Std Dev	CV (%)	Max	Min	Median
dphi (°) in AIR	21.17	0.36	1.70	21.77	20.39	21.07
dphi (°) in NB	22.73	0.60	2.64	23.95	21.80	22.74
Intensity (mV) AIR	55.97	10.54	18.83	88.63	29.53	54.50
Intensity (mV) NB	45.85	15.88	34.63	96.90	21.63	44.05

geometry, showed large variation between individual tubes and between batches. Intensity signals obtained from NB tubes were significantly lower ($p \leq 0.001$, Mann Whitney Wilcoxon Test) than AIR tubes, with median values of 44.05 and 54.50 mV respectively. Intensity values ranged from 29.53 to 88.63 mV in AIR and from 21.63 to 96.90 mV in NB.

Standard deviations for dphi signals were 0.36 for AIR and 0.60 for NB (CV 1.70% and 2.64%, respectively), thus indicating an overall homogeneity in the sensor tubes prepared. On the other hand, higher standard deviations were obtained for intensity signal, being 10.54 mV in AIR and 15.88 mV in NB (CV 18.8% and 34.6%, respectively), indicating a higher variation of this parameter than phase signal. While still useable for respirometric applications, intensity readout is more error-prone and more difficult to interpret and process (Papkovsky et al., 2009). Conversely, phase (or lifetime) signals are internally referenced and therefore remain stable and reproducible despite large variations in intensity signals. A slight once-off decrease in the phase signal when changing from air to NB environment was likely due to swelling of sensor material in water (Borisov, 2018), a known phenomenon affecting the Stern-Volmer constant.

Mean represents the mean value (n = 102), Std Dev represents standard deviations, CV represents coefficient of variation, Max and Min represent the maximum and the minimum values for each group, and Median represents the median values of each group.

In summary, phase signals are more suitable for microbial respirometry and swab testing. Phase signal remained consistent for each swab vial and batch measured. Therefore, one signal threshold can be set and used for all vials. The phase signal threshold of 25 dphi (°) was selected for swab testing application, as it provides reliable detection of all positive samples and eliminates the incidence of false-negative samples. Although Jasionek et al. (2013) used different oxygen probe and detection platform, they also observed that phosphorescence lifetime-based sensing produced more stable and reproducible profiles.

In addition to the parameters described above (intensity and phase signal), the primary optical signal, dphi, can be converted into oxygen concentration. To evaluate the oxygen consumption, we generated an O_2 calibration for a sensor vial by purging it with standard O_2/N2 gas mixtures and taking dphi readings with the FirestingGO$_2$. Thus, the O_2 calibration curve and analytical equation that describes the relationship between O_2 concentration ([O_2], %) and dphi (°) values were established (Fig. 4B): $[O_2] = 0.02*dphi^2 - 2.34*dphi + 62.67$.

Next, we selected one representative respiration profile of a pure culture of *E. coli*, monitored with continuous reading using the relevant option in FirestingGO$_2$. The O_2 calibration equation was applied to the respiration profile curve, converting the dphi values into O_2 concentrations. Fig. 4C shows that the O_2 profile is inverted with respect to the dphi profile. O_2 showed steep changes from air-saturated levels (21%) down to zero levels at around the 5 h time point (TT). This TT value corresponds to signal threshold values of 25° or 18% [O_2], respectively. Although useful for visual representation, the additional conversion of dphi values and profiles into oxygen concentration could bring additional errors and complications in data processing. For routine swab testing, this conversion is unnecessary, so readout of raw phase signal is simpler and more accurate. Altogether, these results prove that sensor vials and the whole sensor-based swab testing system are operating reliably and adequately and that the use of dphi readout is appropriate.

3.4. System calibration and Log(CFU/cm^2) vs TT relationship

Previous calibrations of the O_2 sensor based respirometric assays were conducted under different experimental settings (Jasionek et al., 2013; O'Mahony et al., 2005; O'Mahony and Papkovsky, 2006), namely the type of sensor/probe, assay substrate (microplate or tube), temperature, medium and sample (food homogenate) used, as well as assay readout units (Log (CFU/g) rather than Log (CFU/cm^2)). Therefore, existing calibrations are not valid for the new platform and a new calibration which establishes the relationship between initial microbial load of swabbed surfaces (CFU/cm^2) and measured TT values (in hours) had to be newly generated.

The calibration of the new system was performed using the 10 mL sensor vials and *E. coli* as a model organism for aerobic mesophilic microbiota (Blount, 2015). Serial dilutions (1 in 10) were made directly in the sensor tubes using an overnight culture of *E. coli* and analysed immediately by respirometry (Fig. 5A). The calibration equation was produced by plotting experimentally determined TT values against the known concentrations of *E. coli*, CFU/mL (Fig. 5B). Using the 0.1X factor, as mentioned in section *2.5*, the CFU/mL units can be converted into Log (CFU/cm^2). This gave the following calibration equation for the quantification of microbial load in unknown swabbed samples Log (CFU/cm^2) on the basis of measured TT values with dphi signal threshold of 25°:

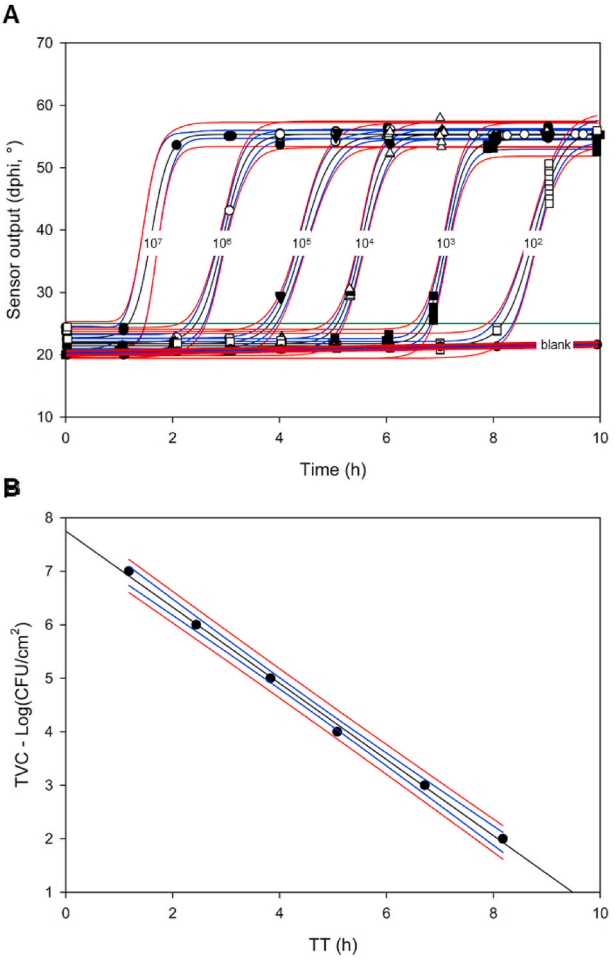

Fig. 5. Respiratory profiles and calibration curve. A: Respiration profiles of serial dilutions of *E. coli* cultures ranging 10^7 CFU/cm^2 – 10^2 CFU/cm^2 in NB in 10 mL sensor vials, along with blank (sterile) samples producing flat profiles. Tubes were incubated at 30 °C and measured hourly with the FirestingGO$_2$ reader. Green line represents the dphi threshold of 25°. B: Time to reach the dphi threshold of 25° (TT, h) for each sensor tube was plotted against the known concentration and fitted with a trendline. The resulting equation was used for the quantification of unknown samples. Black lines represent the non-linear (A) or linear (B) regression lines. Blue lines represent the 95% confidence and the red lines represent the prediction lines.

The corresponding respiration profiles of *E. coli* in Fig. 5A show that at Log (CFU/cm^2) = 7 the result is produced in 1.18 ± 0.17 h while at Log (CFU/cm^2) = 2 the result is produced in 8.18 ± 0.19 h. These initial microbial loads for the assayed surfaces (Log (CFU/cm^2)) were calculated using the equation derived from the calibration curve (Fig. 5B).

3.5. System application

The application of the sensor-based swab testing system was validated by swabbing 100 cm^2 surfaces in plastic trays artificially contaminated (AI) with varying *E. coli* concentrations (10^8 to 10^4 CFU/cm^2) using mini-spray bottles. Pre-wetted swabs were then used to swab the tray top area for the analysis on the sensor system and tray bottom areas for the parallel reference method.

Although pure cultures are useful for mimicking microbial contamination, they are not reflective of the complex microbiota that can be found on real surfaces. Therefore, the second type of artificial contamination (M) was done with meat microbiota, which could be found on surfaces in meat processing plants and on meat samples (Stellato et al., 2017). Different meat cuts were purchased from a local butcher at an open-air market, placed into the 100 cm^2 plastic trays, and incubated at 4° ± 1 °C. The initial analysis was done after 24 h, while for an increased microbial contamination, samples were spoiled for 48 h and 72 h. Following incubation, an imprint of the meat sample on the top tray was done to transfer meat microbiota onto the surface. Subsequently, these pre-wetted swabs were used to sample the footprints of the bottom side and top side (aerobic) of the meat, ensuring that the top and bottom of the plastic trays were swabbed separately.

The third group of samples (S) were obtained by swabbing (according to ISO, 18593:2018) laboratory surfaces like sinks, benchtops etc., to generate a collection of environmental swab samples. The initial microbial load of the samples was calculated using the calibration equation in 3.4 and compared to the microbial load retrieved from the reference method.

The data obtained from both methods was statistically analysed using the present guidelines for method comparison, which define that if the difference between the methods is statistically significant, the alternative method is judged to be different from the reference method (Feldsine et al., 2002). The one-way ANOVA test ($P < 0.05$) showed that there was no statistically significant difference between the two methods ($n = 51$, $P = 0.78$), which can be seen in Fig. 6A. A strong positive correlation (Pearson correlation) between the methods was found ($r = 0.99$, $P < 0.05$). The range of measurements was 0.65–7.87 Log (CFU/cm^2) and in 24 out of 51 (47%) samples, the difference between the two methods (reference vs sensor-based, Δ) was <0.5 Log units, while in 9 samples (18%), the difference was 0.5–1.0 Log units. Meanwhile, eighteen samples (35%), all within the M group, showed a difference greater than 1.0 Log unit, and the maximum value was 2.04 Log unit at 1.57 Log (CFU/cm^2) (sensor) and 3.61 Log (CFU/cm^2) (reference method). The mean difference for the two methods was 0.82 Log unit (absolute value). Considering the individual sampling groups, for *E. coli* contamination (AI) and for naturally contaminated surface samples (S), Δ of <0.5 Log units was observed in 14% of the samples. For the contamination with meat microbiota (group M), Δ of <0.5 Log units was observed in 20% of the samples. The Δ values were in the range 0.5–1 Log units in 2% (AI), 12% (M), and 4% (S) for all analysed samples. Therefore, our data is in agreeance with the "Guideline for Establishing the Suitability of Food Microbiology Methods" (CCFRA, 2001), which indicates that a 5% discrepancy over a 1.0 Log difference in the results is tolerable. Fig. 6B shows the Blant-Altman plot, where the Shapiro-Wilk normality test on the differences in Δ showed that the data were normally distributed ($P = 0.124$), with 100% of the data clustering around

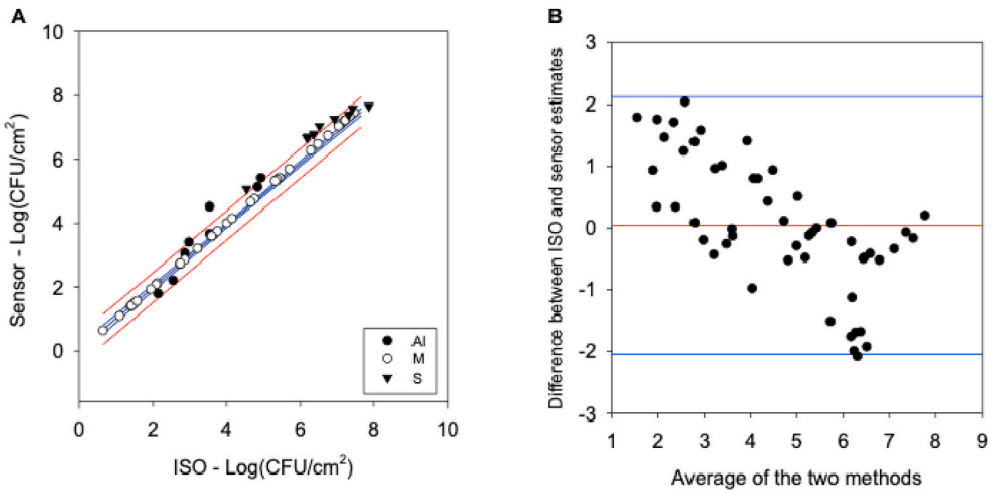

Fig. 6. Application of the sensor swab test to contaminated surfaces. A: Regression plot showing the correlation between the sensor method and the reference ISO method. Swab samples were obtained from artificially contaminated (*E. coli*, group AI) and natural meat microbiota (group M) from naturally contaminated laboratory surfaces (group S). Black lines represent the regression curve, the blue lines represent the 95% confidence, and the red lines represent the prediction interval. B: Blant-Altman plot comparing the difference between the TVC results from the sensor method and the ISO reference method. The red line represents the bias, the blue lines represent the upper and the lower limits of agreement.

the mean of the differences (bias line), and within the limit of agreement of −2.04 and 2.12 Log units. This further confirms the comparability of the sensor-based method with the reference method.

Overall, the sensor-based swab testing system was able to handle not only model contamination with pure *E. coli* cultures, but also contamination with complex meat microbiota and natural (unknown) contamination of surfaces. Furthermore, the new test outperformed the conventional swab TVC test in speed (time to result 2–10 h vs 48–72 h, respectively), simplicity and user-friendliness (no dilutions, real-time readout). Taking into consideration the rapidity, labour-saving characteristics and on-site testing capabilities, the sensor based TVC testing system has clear advantages for surface swabbing, compared to the reference ISO method.

4. Conclusions

The new sensor-based swab testing platform addresses the demand of industry, particularly the food sector, consumers, and society, in rapid, simple, and de-centralised swab testing methods and systems. Unlike other methods, that are generally based on conventional plate counting (ISO, 18593:2018) the system described here provides medium levels of automation, sample throughput and integration, along with modular flexibility, portability, and on-site deployment capability. The cost of goods for such a system for TVC enumeration in swabs is less than $5 k: $1.5–4.5 k for the reader, $300 for the block heater and $1–3 for disposable sensor vials, and this can be further reduced. The whole system can be deployed and used in field conditions with minimal training, at small or large production facilities such as meat plants or environmental labs, i.e., directly where initial swab samples are taken. Its sample preparation procedures remain unchanged and compliant with standard ISO method, thus making the two methods directly comparable and interchangeable. Furthermore, the sensor-based system outperforms the conventional method and has the potential to replace the latter, especially for decentralised and distributed microbiological swab testing. To the best of our knowledge, this is the first portable, quantitative system biosensor-based system developed for on-site swab monitoring of total aerobic microbiota on surfaces. However, the system is limited to oxygen consuming species.

CRediT authorship contribution statement

Elisa Santovito: Methodology, Visualization, Software. **Sophia Elisseeva:** Data curation, Methodology, Writing - original draft, preparation. **Aisling Bukulin:** Data curation, Investigation. **Joseph P. Kerry:** Validation, Writing - review & editing. **Dmitri B. Papkovsky:** Conceptualization, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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