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Opinion

Improving Biopharmaceutical Safety through Verification-Based Quality Control

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Biopharmaceuticals and small-molecule drugs have different approval pathways but the same quality control (QC) paradigm, where the quality of released but untested units is inferred from that of tested but destroyed units. This inference-based QC will likely miss rare prerelease defects, and defects emerging after product release. The likelihood for such defects is heightened for biopharmaceuticals due to their complexity, which makes manufacturing errors more likely, and fragility, which makes postrelease damage more likely. To improve biopharmaceutical safety, we suggest transitioning their QC from inference- to verification-based practice by developing inspection technologies that can nondestructively verify the quality of every vial from the point of release to the point of care. One candidate, water proton NMR (wNMR), is briefly discussed.

Inference- to Verification-Based QC Practice

Drug costs are a huge component of total healthcare costs. High drug prices and unnecessary treatments both elevate healthcare costs [1]. A less recognized factor is defective **drug products** (see Glossary), which refer to products that no longer meet release specifications when administered to patients. The defects might be caused by either manufacturing errors or mishandling during distribution. Defective drug products may render drug therapies ineffective and even cause severe adverse drug events, thereby elevating healthcare costs. The impact of defective drug products is hard to estimate, due to both the scarcity of relevant data (e.g., no data on drug product defects at point of care) and the complexity of the problem (e.g., the social and economic costs of declined vaccination following vaccine-related deaths). Nonetheless, it is known that product defects are a leading cause of drug recalls [2], and drug recalls can be costly [3].

One way to reduce healthcare costs is to minimize defective drug products administered to patients through the best QC practice. Current QC of **biopharmaceuticals** is based on inference, an approach developed for traditional small-molecule drugs. As biopharmaceuticals become ever more complex and their manufacturing modalities become ever more diverse, the inadequacy of **inference-based QC** is becoming increasingly apparent. In this article, we propose to transition QC for biopharmaceuticals from inference- to verification-based practice through technology development and implementation.

Trends

More biopharmaceuticals, including biosimilars, are coming to the market. Many recent approvals are biosynthesized using genetically modified bacteria, yeasts, cells and animals.

Biopharmaceuticals are getting more complex to include live viruses and cells.

More and more biopharmaceuticals are formulated as injection-ready liquids, often at high protein concentration.

The manufacturing of more biopharmaceuticals is outsourced to foreign countries, where FDA oversight is challenging.

The 21st Century Cures Act encourages more data collection by nonexperts in nontraditional clinical trial settings (Real-World Data).

Nondestructive inspection technologies, like wNMR, are being developed.

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Current Drug Product QC Is Based on Inference

Drugs are mixtures of chemicals, and QC of drug products relies on (bio)chemical analysis for testing. On the product side, a common feature is that the drug substance, also called **active pharmaceutical ingredient (API)**, together with excipients and solvents (if any), is enclosed inside sterile sealed containers, such as vials, bottles, and prefilled delivery devices. On the testing side, a common feature is that the container needs to be unsealed so that its content, part or whole, can be transferred to some instruments for testing. Hence, QC testing is destructive from the viewpoint of product integrity. The solution to this conflict between safe distribution, which requires sealing off the product, and QC testing, which requires unsealing the product, is inference-based QC. Below, we use injectables to illustrate this process. The vast majority of biopharmaceuticals approved to date are injectables, supplied either as injection-ready solutions, or as lyophilized powders to be reconstituted into solutions before injection.

Before product release, every vial is subjected to visual inspection for visible defects [4]. Then, a fraction of the vials in every batch is unsealed for QC testing. If data from the tested but unsealed vials meet the release criteria, then the untested but sealed vials in the batch are released. Once released, the only way to detect defects that emerged during distribution is visual inspection, which, by definition, cannot detect invisible defects. This QC process is outlined in Panel A of Figure 1 (Key Figure).

Inference-based QC was developed for small-molecule drugs and is now applied to biopharmaceuticals. It is true that the testing panel of biopharmaceuticals typically is more extensive than that of small-molecule drugs, as the former may have some quality attributes that do not exist in the latter, such as tertiary structure and glycosylation. But regardless of how extensive a specific testing panel is, it is still conducted only on a small fraction of vials in a batch. Hence, for biopharmaceuticals, the quality of released vials is still inferred from the quality of unreleased vials, which are destructed by testing. The vials administered/dispensed to patients have no data on their quality attributes.

For inference-based QC to work for drug products, all vials in a batch should be sufficiently similar when manufactured, so that the quality of a few tested vials can ensure, with high probability, the quality of many untested vials. This requires high-precision manufacturing. Also, all vials in a batch should remain sufficiently similar throughout distribution, so that quality at the point of release can ensure, with high probability, quality at the point of care. This requires damage-free distribution. While prerelease manufacturing errors and postrelease mishandling may happen to all drug products, they pose greater problems for biopharmaceuticals due to their complexity and fragility.

Biopharmaceuticals Are Complex and Biomanufacturing Is not Precise

Biopharmaceuticals are complex. Therapeutic proteins are 50–1000 times larger than small-molecule drugs, and therapeutic viruses and cells are orders of magnitude still more complex than therapeutic proteins. Because of their complexity, most biopharmaceuticals are made through biosynthesis, often using genetically modified organisms. By contrast, most small-molecule drugs are made through chemical synthesis. Drug makers have less control over biosynthesis than chemical synthesis [5]. After biosynthesis, multiple bioprocessing steps are required to extract biopharmaceuticals from host cells [6]. For QC testing, the drug substance is in many cases subjected to bioassays, which typically have larger errors than chemical assays. As a result, biomanufacturing (biosynthesis + bioprocessing + bioassay) is much less precise than chemical manufacturing. Follow-on versions of biopharmaceuticals are designated by regulatory bodies as biosimilars, rather than generics, in recognition that it is impossible, at least to date, for one company to manufacture an exact copy of a biopharmaceutical made by

Glossary

Active pharmaceutical ingredient (API): also known as the drug substance, the component of a drug product that has the intended pharmacological effect.

Biopharmaceuticals: also called biologics, these are biologically derived drugs. There is no unified definition of biopharmaceuticals. They might be molecular entities, including polypeptides (e.g., Symlin), proteins (e.g., Eprex), antibodies (e.g., Avastin), nucleic acids (e.g., Macugen) and polysaccharides (e.g., heparin), or living entities, such as viruses (e.g., Imlygic), cells (e.g., Ducord) or cellularized scaffolds (e.g., Maci). The vast majority of marketed biopharmaceuticals are polypeptides, proteins, and antibodies. In terms of medical indications, biopharmaceuticals might be therapeutics (e.g., Avastin), diagnostics (e.g., NeutroSpec), or vaccines (e.g., Flublok). Some biopharmaceuticals are extracted from natural sources (e.g., Hizentra from human blood, and heparin from porcine intestines), while some are chemically synthesized (e.g., Symlin, and Macugen). However, most biopharmaceuticals approved recently are manufactured biologically using genetically modified bacteria (e.g., Lantus), yeasts (e.g., Victoza), insect cells (e.g., Flublok), plant cells (e.g., Elelyso), mammalian cells (e.g., Avastin by rodent cells, and Elaprase by human cells) or even animals (e.g., ATryn by GM goats).

Drug product: the entity received by the patient, which includes not only the drug substance, but also excipients and solvents, sealed inside a labeled container.

Inference-based QC: a mode of QC where the quality of one population is inferred from data collected from another population, or the quality at one time point is inferred from data collected at another time point. Current drug product QC is inference-based.

Longitudinal relaxation time T_1 and rate R_1: characteristic time of the magnetization recovery along Z-axis (coinciding with the direction of external magnetic field of NMR instrument). Relaxation rate R_1 is the inverse of longitudinal relaxation time T_1 ($R_1 = 1/T_1$). In most cases, T_1 is defined by molecular motion, thus is

another company. In fact, quality attributes of a biopharmaceutical made by the same company may drift over time, for example, a company may have difficulty making the exact copy of a biopharmaceutical it made, say, a decade ago [7]. Perhaps less widely recognized is that manufacturing imprecision can lead to larger unit-to-unit variability for biopharmaceuticals than for small-molecule drugs. In essence, every vial is different.

Unit-to-unit variability caused by manufacturing imprecision is nothing new; it happens to small-molecule drugs too. A 1962 paper by industry scientists entitled 'Acceptance sampling of finished drug products' pointed out that: 'No two capsules, tablets or ampules will contain exactly the same amount of ingredient . . . .The difference is not one of kind – only of degree' [8]. Indeed, a study published in 1961 found that tablets of three small-molecule drugs from the same bottle in some cases display potency variability outside the pharmacopeia-permitted range for these drugs (±5%) [9]. Such tablet-to-tablet variability reflects the imprecision of drug-product manufacturing. Even today, incorrect potency is one of the most common reasons for drug recalls [2].

Compared to small-molecule drugs, biopharmaceuticals likely have even higher unit-to-unit variability due to their higher level of manufacturing imprecision, which is duly recognized in the United States Pharmacopoeia (USP). For example, for small-molecule oral drugs, the acceptable potency variability is 90–110% of claimed potency (95–105% in some cases) [10]. By contrast, for epoetin products (a glycoprotein), the acceptable potency variability is 80–125% of claimed potency [11]. Like small-molecule drugs, some released epoetin products have average potency outside this pharmacopeia-permitted range [12,13]. In addition to potency, other quality attributes may also display unit-to-unit variability, such as subvisible particle content [14]. Unit-to-unit variability (within the same batch) increases the number of units to be destructively tested in order to achieve statistical significance.

Biopharmaceuticals Are Fragile and Defects May Emerge after Product Release

Biopharmaceuticals are fragile, which is a consequence of their complexity. Most therapeutic proteins require tertiary structures for their bioactivities. The tertiary structures of proteins are held together mostly by intramolecular noncovalent interactions with a net stability on the order of just a few RT (thermal energy, which is 2.5 kJ/mol at 25°C) [15]. Viruses and cells are assembled from proteins and other biomacromolecules, which are held together mostly by intermolecular noncovalent interactions. By contrast, the bioactivity of most small-molecule drugs relies on neither intramolecular noncovalent interactions (as in proteins) nor homo- and hetero- intermolecular noncovalent interactions (as in viruses and cells). For small molecule drugs, bioactivity depends on the covalent bonding between its constituent atoms. To damage small-molecule drugs, breakage of covalent bonds is necessary. To damage biopharmaceuticals, breakage of noncovalent interactions suffices. As is well known in chemistry, the breakage of noncovalent interactions requires much less energy (<20 kJ/mol in most cases) than that of covalent bonds (>200 kJ/mol in most cases).

The fragility of biopharmaceuticals makes them susceptible to damage by physical stresses commonly encountered during distribution, such as agitation, heating, freeze/thaw, and sunlight. Physical stresses can perturb the tertiary structures of proteins, leading to partial unfolding, often followed by aggregation. If the concentration is sufficiently high, protein aggregation may even occur without any apparent stress [16]. Partially unfolded proteins may have reduced bioactivity while aggregated proteins may have heightened immunogenicity (Box 1). Further, metal-induced protein aggregation may occur even without any additional stress [17,18]. Hence, new defects emerging during distribution is a much bigger concern for biopharmaceuticals than for small molecule drugs.

dependent on diffusion and on the interaction of a molecule with environment (lattice), hence often also called *spin-lattice relaxation time*.

Magnetic resonance imaging (MRI): a radiology technique that can obtain images of anatomy, inner organs, and various dynamic processes, such as blood oxygenations, in a human body; it can detect a number of disorders and/or pathologies. NMR forms the scientific basis for MRI, and since water protons are by far the largest population of detectable nuclei in the human body, MRI images mostly reflect the distributions and interactions of water in the human body.

NMR: a powerful analytical spectroscopic technique based on the magnetic properties of atomic nuclei in a molecule possessing nuclear spin (such as ^{1}H, ^{13}C, ^{15}N, and ^{31}P). It is widely used to study the structure, dynamic properties, environment, etc. of molecules.

Proton exchange: the process of exchange of labile (dissociative) protons between molecules. In aqueous protein solutions, it is mainly an exchange between water protons and labile protons in proteins (e.g., $–NH_2$, –OH, –NH–CO–, etc.). Proton exchange significantly affects transverse relaxation time T_2 of water protons.

Transverse relaxation time T_2 and rate R_2: characteristic time of the magnetization loss along X,Y-plane (perpendicular to the direction of external magnetic field of NMR instrument). Relaxation rate R_2 is the inverse of transverse relaxation time T_2 ($R_2 = 1/T_2$). T_2 is define by the decoherence of different nuclear spins in a molecules, hence often is also called *spin-spin relaxation time*. T_2 is sensitive to molecular motion and also to local transient magnetic fields due to inhomogeneity of various nature as well as to chemical exchange such as **proton exchange**.

Real-World Data (RWD): data relating to patient health status and/or the delivery of health care routinely collected from a variety of sources (https://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm513027.pdf).

To minimize product damage during distribution, drug makers provide detailed instructions on proper product handling, such as avoiding shaking and sunlight, and keeping the product refrigerated at 2–8°C without freeze/thaw (cold-chain). However, it is hardly possible to avoid shaking and sunlight completely. Also, maintaining cold-chain integrity throughout distribution is challenging even in developed countries [19]. A report released in 2012 by the Office of Inspector General of the US Department of Health and Human Services found that vaccines for children 'stored by 76 percent of 45 providers we reviewed were exposed to inappropriate temperatures' and 'all 45 providers recorded temperatures that differed from our independently measured temperature' (http://oig.hhs.gov/oei/reports/oei-04-10-00430.pdf). This report provides a glimpse of the reality of cold-chain integrity in drug-product distribution.

Anticipating that some physical stresses are inevitable during distribution, drug makers stabilize biopharmaceuticals with excipients. For example, Zn^{2+} is commonly used in the formulation of insulin products; it can stabilize insulin against heat-induced unfolding [20]. Drug developers may use protein engineering to remove vulnerable spots. For example, in unglycosylated human interferon-β products (Betaseron and Extavia), Cys-17 is mutated to Ser-17 to minimize disulfide crosslinking-induced aggregation [21]. Drug makers also conduct stability testing in accordance with ICH Q1A (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM073369.pdf) to demonstrate that their products can withstand certain stresses. However, drug makers have little control over product distribution, and stability testing conducted under controlled settings cannot encompass all postrelease stress scenarios, which may vary greatly among individual units. Drug units in the same batch might be shipped to different locales via different transportation modes, stored for different durations, dispensed by different hospitals and pharmacies, and finally administered by different end-users, including self-administration by patients.

The combination of product fragility and variable distribution history may lead to greater unit-to-unit variability at point of care than at point of release, to the extent that some units no longer meet release specifications, that is, they become defective during distribution. The defects might be invisible (e.g., subvisible protein aggregates). Without quantitative postrelease QC testing, the defective units will likely go undetected and be passed onto patients, causing harm in some cases.

Examples of Recent Inference-Based QC Failures for Biopharmaceuticals

The above analysis shows that the fundamental premises of inferenced-based drug-product QC, namely drug manufacturing is precise enough and drug distribution is fail-proof enough, are less applicable to biopharmaceuticals than to small-molecule drugs. Below are several recent examples of inference-based QC failures for biopharmaceuticals. Cases 1–5 involve defective products. In Case 6, the cause of deaths is unclear and this uncertainty led to public panic.

Case 1: Suspension of Flu Vaccines in Europe and Canada in 2012

In October 2012, the World Health Organization (WHO) announced that six European countries and Canada suspended several influenza vaccines due to visible protein aggregates (http://www.euro.who.int/en/health-topics/communicablediseases/influenza/news/news/2012/10/precautionary-suspension-of-novartis-influenza-vaccine-in-some-european-countries). About the same time, the UK Medicines and Healthcare products Regulatory Agency (MHRA) requested Novartis Vaccines and Diagnostics to recall two batches of the influenza vaccine Agrippal (http://www.sehd.scot.nhs.uk/publications/DC20121031drugalert34.pdf). There was no report of harm and the suspension was lifted in some countries shortly after (http://www.euro.who.int/en/health-topics/communicable-diseases/influenza/news/news/2012/11/use-of-novartis-influenza-vaccines-resumed-in-some-countries).

Verification-based QC: a mode of QC where the quality of one population at a given time point is based on data collected from that population at that time point. Airport security screening is an example of verification-based QC, where all passengers are checked right before boarding.

wNMR: NMR parameters of the water protons, which include the signal intensity $I(^1H_2O)$, the chemical shift $\delta(^1H_2O)$, the diffusion coefficient $D(^1H_2O)$, the **longitudinal relaxation rate** $R_1(^1H_2O)$, and the transverse relaxation rate $R_2(^1H_2O)$. Of these parameters, $R_1(^1H_2O)$ and $R_2(^1H_2O)$ can be obtained in a straightforward manner using time-domain NMR spectrometer.

Key Figure

Inference- versus Verification-Based QC

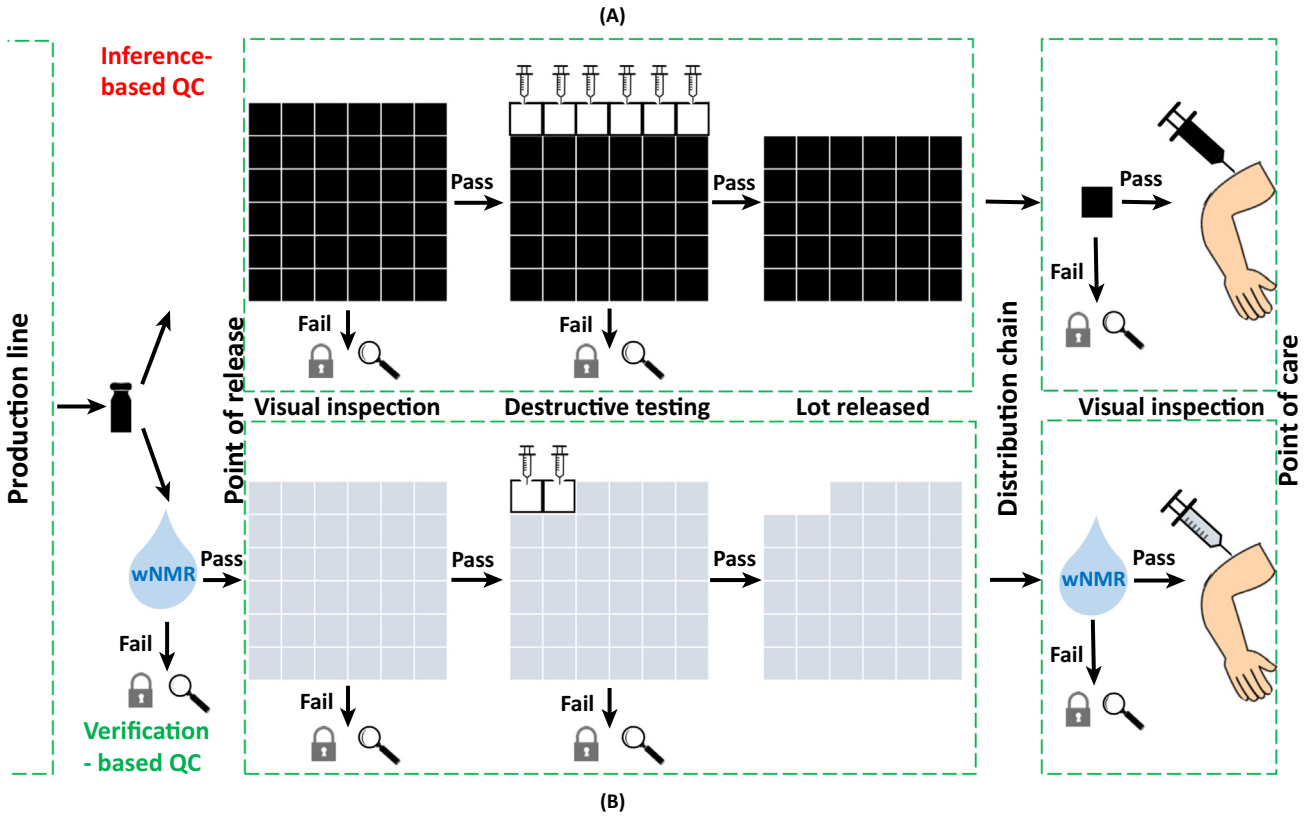

Figure 1. For a Figure360 author presentation of Figure 1, see the figure online at http://dx.doi.org/10.1016/j.tibtech.2017.08.010#mmc1.
(A) Inference-based QC of biopharmaceuticals: in practice, there are certain variations of this outline. For example, visual inspections might be performed more than once [76]. Also, drug makers may collect released vials for testing. These variations do not change the fact that vials administered to patients have no data to ascertain their quality. (B) Verification-based QC of biopharmaceuticals using wNMR: noninvasive technologies like wNMR make it possible to collect data on every vial. Collecting data on every vial ensures batch uniformity by removing outliers at both point of release and point of care. Black squares denote the units with no data; blue squares denote the units with partial data on them. The lock and magnifying glass pictograms denote the units rejected/sent for extensive analysis. Abbreviations: QC, quality control; wNMR, water proton NMR.

Among the six cases presented here, this is the only one not linked to any reported adverse drug events. What sets this case apart from others is that the product defect was visible and hence was caught early. It is much harder for inference-based QC to catch invisible defects.

Case 2: Recall of Novomix 30 in Europe in 2013

Novomix 30 contains insulin aspart, a human insulin analog for treating diabetes. It is formulated as a white suspension containing 30% soluble insulin aspart and 70% protamine crystallized insulin aspart, presented in prefilled delivery devices. On October 25, 2013, 33 batches (3.3 million cartridges) of insulin Novomix 30 were recalled. The press release from the European Medicines Agency (EMA) states that 'a manufacturing problem during the filling of the

Box 1. Protein Aggregation in Biopharmaceuticals and Challenges for QC

Proteins are large molecules with multiple amino acid side chains. These side chains can lead to both intramolecular interactions, which stabilize the tertiary structures of proteins, and intermolecular interactions, which are responsible for protein aggregation. Protein aggregation may involve both covalent and noncovalent interactions. Partially unfolded protein molecules are prone to aggregate [61]. Aggregation of human endogenous proteins is associated with neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases [62,63]. Aggregation of proteins in biopharmaceuticals may diminish efficacy and induce immunogenicity [53]. Several cases cited in the main text involve some aspects of protein aggregation. Minor changes in formulation may have a big impact on protein aggregation. For example, Omontys has two formulations; the one containing phenol, a preservative, has much higher amount of subvisible protein aggregates than the one containing no phenol [51]. Another example is Referon-A, where replacing human serum albumin with the surfactant polysorbate-80 led to significant reduction of protein aggregation and patient immunogenic response [64,65].

Current trends in the dosage forms and presentation formats of biopharmaceutical products, designed to facilitate drug administration and patient compliance, may elevate the risk of protein aggregation. More and more biopharmaceuticals are formulated as aqueous solutions ready for injection, often for patient self-injection via the s.c. route. For s.c. injection, drug makers tend to limit the injection volume to $<$1.5 ml. This volume restriction may result in high protein concentration in the final product ($>$25 mg/ml is common for antibody products [66]). Simple physicochemical principles dictate that high protein concentration promotes aggregation. Also, a growing number of biopharmaceuticals are presented in prefilled delivery devices to facilitate injection [67]. Lubricant droplets and other particles in such devices may act as nuclei for protein aggregation, leading to higher levels of aggregation than in traditional glass vials in some cases [17,68].

Protein aggregation poses unique challenges to biopharmaceutical QC in several ways. First, the size of aggregates that might pose problems may vary significantly from product to product, even for products within the same class. For example, for regular insulin (human, porcine, and bovine), aggregates as small as dimers may induce insulin antibody in some diabetic patients [69]. By contrast, the long-acting insulin, Lantus, forms micron-sized aggregates at physiological pH [70], but is widely used. Indeed, there is much uncertainty surrounding the impact of protein aggregates on the safety and efficacy of biopharmaceuticals [53]. The wide size range of protein aggregates (from nm to μm) poses a huge technical challenge to their detection and quantification [14,54].

Second, the protein aggregate level may display unit-to-unit variability, both at the point of release, and probably even more so at the point of care. The possibility of within-batch variability of subvisible protein aggregates has been pointed out in an article by industry scientists [14].

Third, the protein aggregate level in a vial is not static, but might increase over time. At the point of care, the aggregate level in some vials might exceed the release specification and pose danger to patients. Case 5 is one example [35]. Monitoring such evolving product attributes is challenging.

Finally, different patients may have different tolerance toward protein aggregates. As early as 1987, it was found that insulin dimers induce insulin antibodies in some but not all patients [69]. Case 5 is another example [35].

cartridges, which resulted in some batches of NovoMix 30 containing too high or too low amounts of insulin units per millilitre' (http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2013/10/news_detail_001933.jsp&mid=WC0b01ac058004d5c1). It further states that 'only a small percentage of cartridges (0.14%) contain a wrong amount. However, in the affected cartridges the level of insulin may vary between 50% and 150% of the labelled insulin units, which could lead to hypoglycaemia or hyperglycaemia.' Indeed, 1 month before the recall, on September 27, 2013, a patient collapsed at home in a hypoglycemic state after taking a product that belongs to one of the recalled batches of Novomix 30. Subsequent test confirms that her insulin dose was indeed incorrect. According to the FDA website: 'The patient was immediately taken by emergency ambulance to the hospital. While in hospital, the patient suffered two more hypoglycaemic episodes and went into coma.' The patient eventually recovered after medical treatment. (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/detail.cfm?mdrfoi__id=3621493).

Protein concentration cannot be quantified by visual inspection and therefore the defect of Novomix 30 has to be detected by destructive quantitative testing. To detect a defective rate of

0.14% with 90% confidence, one needs to subject almost 40 000 out of 100 000 units for destructive testing (see Statistical Analysis in the Supplemental Information online). This is unrealistic. This case illustrates the difficulty to catch rare pre-release defects with inference-based QC.

Case 3: Clusters of Eprex-Associated Pure Red Cell Aplasia in Asia in 2006 and 2013

Epoetin (Eprex and Epogen) is recombinant human erythropoietin for treating anemia. It is formulated as a liquid, presented in vials and prefilled syringes. Patients who develop anti-erythropoietin antibodies suffer a life-threatening condition called pure red cell aplasia (PRCA), with some patients requiring kidney transplantation [22]. PRCA is a rare condition and spikes of cases are considered a drug-safety signal, which may lead to certain restrictions on product distribution and usage.

After a surge of PRCA cases in Thailand between 2004 and 2007, a study examined the protein aggregation levels in Eprex [23]. This study found that in hospital pharmacies, all of the examined units had the protein aggregation levels within the release specification. But in retail pharmacies, 27% of the examined units had the protein aggregation levels above the release specification; in smuggled products, this number was 58%. The maker of Eprex subsequently stopped sales of Eprex through retail pharmacies in Thailand in 2006. The cause was attributed to breaches of the cold chain [23].

Considering that maintaining cold-chain integrity is challenging [19], it is conceivable that cold-chain breaches might be a contributing factor in other adverse drug events associated with biopharmaceuticals. In 2012–2013, there was a cluster of Eprex-associated PRCA cases in Singapore, including fatalities [24]. This incident prompted local health authorities to contraindicate Eprex for subcutaneous (s.c.) administration in chronic kidney disease patients. The statement released by the Singapore Health Sciences Authority includes the following reminder: 'Healthcare professionals are reminded to ensure that Eprex and other ESAs are stored between 2°C and 8°C (as stated in their package inserts) and to advise their patients on the appropriate storage/handling of these ESAs (www.hsa.gov.sg/content/hsa/en/Health_Products_Regulation/Safety_Information_and_Product_Recalls/Product_Safety_Alerts/2013/contraindication_of.html).

Case 4: Recall of Repackaged Avastin in US in 2013

Avastin (bevacizumab) is a monoclonal antibody and is formulated as liquid presented in glass vials. Avastin is approved for treating cancer, but is used off-label for certain eye conditions such as age-related macular degeneration [25] and diabetic macular edema [26]. To that end, Avastin is repackaged into plastic syringes and administered to the eye through intravitreal injection.

In 2013, there was a nationwide recall in the US of Avastin repackaged by a compounding pharmacy (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/detail.cfm?mdrfoi__id=3621493). The recall was prompted by reports of patients experiencing vision loss after receiving intravitreal bevacizumab (IVB). There are other reports of IVB-related vision loss in several countries; some were due to microbial infection [27,28], while others were due to sterile inflammation with unclear etiology [29–31]. It was found that some repackaged Avastin syringes contained high levels of protein aggregates [32,33]. Whether protein aggregates contributed to sterile inflammation observed in some patients is not clear.

Case 5: Termination of an Epoetin Biosimilar Clinical Trial in Asia and Europe in 2009

In 2009, a clinical trial was conducted at 89 medical centers in Asia and Europe for s.c. injection of an epoetin biosimilar (HX575). Of the 174 patients receiving HX575, two (1.1%) developed

neutralizing antibodies against erythropoietin. Of the two, one developed PRCA while the other died of myocardial infarction. Because of these two events, the clinical trial was terminated on June 9, 2009 [34]. It took 7 years and multiple studies for the matter to be clarified [17,34,35].

Follow-up studies revealed that the adverse events were caused by a combination of patient and product factors [35]. On the patient side, only patients carrying certain HLA alleles developed neutralizing antibodies against erythropoietin. On the product side, some syringes of HX575 retrieved from clinical trial centers contained unusually high levels of epoetin aggregates. Epoetin aggregation in HX575 was caused by tungsten particles shed in the manufacturing of prefilled syringes. The level of tungsten varied from syringe to syringe, which explains the syringe-to-syringe variability of epoetin aggregate levels [17]. Also, it took >6 months for the aggregates to rise to significant levels, which explains why the defect was missed by release inspection. This is an example of the drug substance interacting with the primary packaging material, leading to postrelease product defects in some, but not all vials. The only way to catch such defects is point-of-care QC testing of every vial before injection.

Cases 6: Suspension of Flu Vaccine Fluad and the Ensuing Panic in Italy in 2014

In November 2014, three elderly people in Italy died within 48 h after receiving the influenza vaccine Fluad. This prompted the Italian Medicines Agency to suspend two batches of this vaccine on November 27, 2014. One week later, the EMA excluded a causal link between vaccination and the reported deaths (http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2014/12/WC500177992.pdf). Four weeks later, on December 23, 2014, based on negative toxicity and sterility test results, the two batches were reintroduced [36]. Due to extensive media coverage, this suspension caused a panic in Italy about vaccination [36]. Some suggested that the 12% decrease in vaccination rate in 2014 might have contributed to the 9.1% increase in mortality rate in 2015 in Italy [37].

The cause of the three deaths is still unclear. The deaths might have been entirely coincidental and have nothing to do with vaccination. Alternatively, they might have been due to patient factors, that is, the three patients were not suited for vaccination [38,39], or they might have been due to product factors, that is, the vials of vaccine administered to the three patients were defective, or both (see Case 5). To fully verify or refute a causal link between the reported deaths and vaccination, data on both the patient (was this patient suited for vaccination?) and the drug (was this vial of vaccine defective when administered?) are probably necessary. In the absence of such data, the conclusion that there was no causal link between the reported deaths and vaccination was almost unavoidable. The lesson of this case is not that defective vaccines caused deaths. Rather, the lesson is in the absence of relevant data, the cause of vaccine-related deaths remains unknown and this uncertainty undermines people's confidence in vaccination.

Time to Transition Biopharmaceutical QC from Inference- to Verification-Based Practice

Biopharmaceuticals and small-molecule drugs have separate approval pathways, in recognition of their many distinctions [40]. However, they have the same QC paradigm, which is based on inference. The six cases presented above highlight the inadequacy of inference-based QC for biopharmaceuticals.

One might argue that the six cases are isolated incidents and do not represent biopharmaceuticals in general. But systematic comparisons of biopharmaceuticals and small-molecule drugs support the conclusion that inference-based QC is working less well for biopharmaceuticals. Between 2004 and 2013, the average annual recall rate in the US was 2.7% for biopharmaceuticals and 2.0% for small-molecule drugs [41]. For drugs approved by the FDA

between 2001 and 2010, every year from 2001 to 2015, a higher percentage of biopharmaceuticals was associated with postmarket safety events than that of small-molecule drugs [42]. Of course, not all recalls and postmarket safety events are caused by defective drug products, but these numbers do suggest that biopharmaceuticals, on average, have more safety issues than small-molecule drugs. Also, keep in mind that these two comparative studies do not include biosimilars. The FDA approved the first biosimilar in 2015; many more are in the pipeline (http://www.biosimilarspipeline.com). With biosimilars, the challenge to ensure biopharmaceutical quality becomes stiffer. Case in point: the availability of biosimilar epoetin products in Thailand led to a surge in cases of PRCA; a potentially fatal condition [43].

It is advisable that the biotech community takes proactive actions to pre-empt public backlashes against biopharmaceuticals. Once an unfavorable public opinion is solidified, it is hard to reverse it. As shown by the controversies about genetically modified (GM) foods [44] and vaccines [39], once the public becomes distrustful of a category of products, the entire sector suffers and the public collectively gains no benefit either. This is in spite of the fact that GM foods and vaccines have helped countless people around the world. The best way to prevent such backlashes is to ensure product safety through best QC practice.

With this in mind, we suggest that biopharmaceuticals and small-molecule drugs adopt separate QC paradigms, in parallel to their separate approval pathways. Specifically, we suggest **verification-based QC** for biopharmaceuticals, which means quantitative inspection of all vials in a batch, from the point of release to the point of care. Verification-based QC does not contradict quality by design (QbD) for drug manufacturing (https://www.fda.gov/downloads/drugs/guidances/ucm073507.pdf), in the same way that health insurance does not contradict healthy lifestyle. Even with QbD, there will always be defective units both before and after release. Verification-based QC adds another layer of quality assurance. Additionally, in on-demand drug production [45,46] or individualized drug therapy [47,48], the number of doses made might be so small that inference-based QC cannot achieve statistical significance (see Supplemental Information online), and therefore verification-based QC is the only option.

Potential Enabling Technologies for Verification-Based QC

Verification-based QC requires enabling technologies. The first and foremost requirement of such technologies is nondestructiveness, that is, the ability to collect quantitative data on biopharmaceuticals without damaging/tampering the product in any shape or form. The inspection has to rely on electromagnetic or acoustic waves to glean information from the sealed drug product, regardless of the size, shape and optical opaqueness of the container. This requirement alone is demanding. To acquire data on every vial (100% quantitative inspection), there are additional requirements.

For 100% point-of-release inspection, high-throughput capacity, at a speed comparable to current 100% visual inspection, which is ~30 s/vial [4], is necessary. For 100% point-of-care inspection, ideally, the user should be able to scan the vial with a handheld device and within seconds obtain a binary answer: yes (for injection) or no (for no injection). Afterwards, the data are automatically recorded and transmitted to relevant parties [doctors, pharmacies, drug makers, payers, FDA, Centers for Disease Control (CDC), etc.].

These requirements collectively pose a huge technical challenge. Table 1 lists a few potential options. Of the five technologies listed in Table 1, small-angle X-ray scattering and high-resolution ultrasonic spectroscopy might damage the API due to their high energy. In fact, USP chapter 787 states that for therapeutic protein injections 'Sonication should be avoided' [49]. Therefore, they are of limited value for drug product inspection. This leaves nuclear magnetic resonance (**NMR)**, near infrared (NIR), and Raman spectroscopy.

Table 1. Characteristics of Potential Nondestructive Analytics for Verification-Based QC[a]

	Method	Wavelength[b] (m)	Can penetrate nontransparent container?	High-throughput compatible?	Point of care suitable?	Information Source	
						H_2O	Solute
Electromagnetic wave	NMR	10^0 to 10^1	Yes	Yes	Yes	Yes	Yes
	NIR	10^{-7} to 10^{-6}	No	Yes	No	Yes	Yes
	Raman	10^{-7} to 10^{-6}	No	Yes	No	Yes	Yes
	SAXS	10^{-11} to 10^{-10}	Yes	No	No	No	Yes
Acoustic wave	HR-US	10^{-6} to 10^0	Yes	No	Yes	No	Yes

[a]Abbreviations: HR-US, high-resolution ultrasonic spectroscopy; NIR, near infrared spectroscopy; SAXS, small-angle X-ray scattering; NMR, nuclear magnetic resonance.
[b]The wavelength range is approximate.

Typical spectroscopic analysis is based on signals from the solutes. For biopharmaceuticals, the solute signals are complex [50]. Also, the analyte signals in NMR, NIR and Raman spectroscopy all face interference from the water signal to some extent, which further complicates data collection and analysis. Hence, chemical analysis based on the solute signals has limited applicability in nondestructive product inspection, which requires high speed and simplicity.

In this regard, medical imaging is instructive. The primary goal of noninvasive medical imaging is to detect abnormality, rather than specifying biochemical and histological details underlying the abnormality, which can be identified by subsequent invasive procedures such as biopsy. The same can be said about nondestructive drug-product inspection; the primary goal of which is to detect product defects, rather than specifying the chemical details of the defects, which can be identified by subsequent destructive testing, such as size-exclusion chromatography and mass spectrometry.

Recognizing the proper goal of nondestructive QC inspection liberates one from using the API as the signal source for inspection. Instead, one might use water as the signal source. The rationale for using water as the signal source is straightforward: water is typically 10^3–10^6 more concentrated than the API, and therefore its signal is more intense. In this regard, medical imaging is again instructive; in **magnetic resonance imaging (MRI)**, the signal source for detecting abnormality is primarily water rather than proteins or other biomacromolecules. In the context of biopharmaceutical solutions, the water signal is simpler and stronger than the protein signal, so switching the signal source from the API to water simplifies data acquisition and processing. Also, water has extensive interactions with the API and excipients. Therefore, product defects may indeed be reflected in the water signal, although the chemical details of the defects are likely to remain hidden. Another key advantage of using the water signal is that the inspection protocol will be applicable to all biopharmaceutical solutions with little variation from product to product.

Role of Verification-Based QC

Nondestructive technologies such as **wNMR** (Box 2) cannot replace destructive technologies, the same way that MRI cannot replace biopsy. Verification provided by nondestructive technologies is partial verification, that is, mere detection of abnormalities. In essence, the water signals serve as vital signs of the drug product (see Supplemental Information online).

Verification-based QC is for drug products and not drug substance. Many critical quality attributes of a drug product, such as amino acid sequence, glycosylation, bioactivity, and contaminants (host cell DNA, endotoxin, metal ions, etc.), can and should be tested at the drug-substance stage using invasive technologies. Verification-based QC is used to detect manufacturing errors likely occur at the fill-finish stage, such as wrong concentration as in Case 2, or defects likely emerge after product release, such as protein aggregation as in Cases 3 and 5. The role of verification-based QC is to use 100% data collection to verify prerelease product uniformity and postrelease product integrity. This is depicted in Panel B of Figure 1.

Other Benefits of Point-of-Care QC Data

Nondestructive inspection technologies like wNMR make it possible to collect quantitative data on every vial of a biopharmaceutical product in its entire life cycle. Such data have the potential to reduce the uncertainty in areas beyond product quality. Here are a few examples.

When responding to reports of adverse drug events, knowing whether the reactions are caused by defective product units or normal product units can make a difference. In Case 5, had it been known that the two adverse events were caused by a combination of patient genetic characteristics and defective drug units, the clinical trials might not have been terminated. In Case 6, knowing whether the deaths were caused by defective vaccine products might have reduced the level of panic over vaccination. Another example is Omontys, where the drug was withdrawn before it was found that one version of it had a higher level of protein aggregates than another version [51]. Had this fact been known at time of decision, a total withdrawal of both versions might have been avoided [52].

Box 2. wNMR for Verification-Based QC

wNMR provides a way to assess the quality of biopharmaceuticals in a speedy and nondestructive fashion. The various NMR parameters of ^1H_2O serve as vital signs for a vial of biopharmaceutical solution. Of the various wNMR parameters, the **transverse relaxation rate** of water, $R_2(^1H_2O)$ (Figure IA), is most sensitive to protein concentration and aggregation (see Supplemental Information online), and hence is discussed here.

As shown in Figure I, in the absence of protein aggregation, $R_2(^1H_2O)$ grows linearly with protein concentration. However, such linearity ceases to exist if the protein solution is stressed (e.g., by heating) resulting in the formation of subvisible aggregates (Figure IB) [71]. Of note, both of the observed trends could be used to detect the deviations in the quality of a biopharmaceutical formulation based on the known/expected $R_2(^1H_2O)$ values. However, as seen from Figure IB, once the deviation from the expected value of $R_2(^1H_2O)$ is detected, it is not feasible to define the source of the defect — intolerable level of protein aggregates (② in Figure IB) or unacceptable deviation of protein concentration (① in Figure IB). Nevertheless, $R_2(^1H_2O)$ does allow one to immediately detect defective units, whatever the cause of such defects, be it wrong concentration (as in Novomix 30), or aggregation (as in Eprex).

In light of the importance of controlling protein aggregate level in biopharmaceuticals, we investigated this in some detail. Using bovine serum albumin and human γ-globulin, we demonstrated that $R_2(^1H_2O)$ correlates with aggregate size determined by dynamic light scattering techniques [72]. Using human insulin, it was demonstrated that $R_2(^1H_2O)$ can detect aggregates even when the protein concentration is low (0.4 mg/ml) [73].

Of special importance is the application of $R_2(^1H_2O)$ to study the stability of formulations of therapeutically relevant proteins, such as monoclonal antibodies (mAbs). A current trend in the pharmaceutical industry uses highly concentrated mAbs formulations, about 50 mg/ml and above. Therefore, it is also critical to test sensitivity of $R_2(^1H_2O)$ within this concentration range where aggregation is known to result in a wide range of particle sizes — from subvisible to visible aggregates, and from soluble to insoluble ones. It is also meaningful to study mAb stability to a number of different stresses typically encountered by biopharmaceutical formulations during distribution, such as freeze–thaw, heating, and agitation. We have shown that $R_2(^1H_2O)$ is sensitive to the formation of aggregates generated by all three above stresses [74]. Removal of ≥5 μm particles, and ≥0.45 μm particles by sequential filtration of the stressed solutions results in a corresponding gradual decrease in $R_2(^1H_2O)$. After two sequential filtrations, $R_2(^1H_2O)$ remains larger than unstressed controls, demonstrating sensitivity to small soluble aggregates [74]. Thus, compared to conventionally used analytical techniques (size-exclusion chromatography, dynamic light scattering, and micro-flow imaging), $R_2(^1H_2O)$ is shown to be the most consistently sensitive to both insoluble and soluble aggregates, and visible and subvisible particles.

In addition to biopharmaceuticals, $R_2(^1H_2O)$ can also be used for QC of nanomedicines. This potential was demonstrated using polystyrene nanoparticles (NPs) [75]. As these NPs have no exchangeable protons, the sensitivity of $R_2(^1H_2O)$ toward their aggregation comes from a different mechanism; namely the compartmentalization of water molecules (see Supplemental Information online). $R_2(^1H_2O)$ is also sensitive to surfactant micellization [72].

In summary, wNMR provides the prospective means to conduct quantitative nondestructive inspection of drug products. We term this technology 'wNMR', and it has a lot of similarities with MRI, such as the signal source, water. However, unlike MRI, wNMR does not require 3D magnetic field gradients for its operation and hence can be carried out using a simple benchtop time-domain NMR spectrometer with a wide bore, capable to accommodate sealed drug vials. The simplicity of this measurement makes it possible to use wNMR to conduct 100% quantitative QC inspection at both point of release and point of care. This process is illustrated in Panel B of Figure 1 in main text.

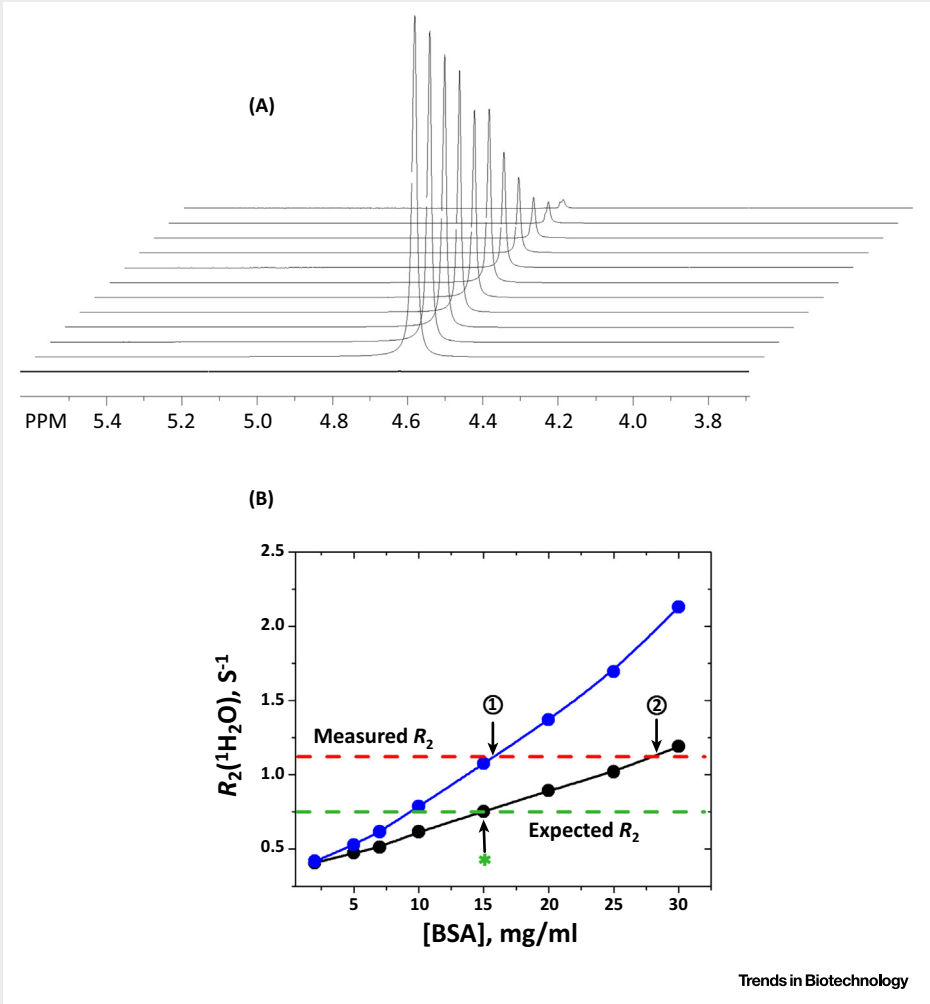

Figure I. $R_2(^1H_2O)$ Measurements and Interpretations. (A) Carr–Purcell–Meiboom–Gill experiment for observation of $R_2(^1H_2O)$ in single transient acquisition experiment. (B) Dependence of $R_2(^1H_2O)$ on BSA (PBS buffer, pH 7.4) concentration (black) and after the stressing of BSA solutions by heating for 10 min at 60 °C (blue). Red and green broken lines model the observations in quality control experiment, observed $R_2(^1H_2O)$ value (red line) and expected $R_2(^1H_2O)$ value for 15 mg/ml (green star) BSA solution (green line). Two extreme scenarios follow from this measurement: ① correct concentration, but aggregated; ② no aggregates, but incorrect concentration. Adapted, with permission, from [68,69]. Abbreviations: BSA, bovine serum albumin; $R_2(^1H_2O)$, transverse relaxation rate.

Point-of-care data may contribute to our understanding of therapy outcome variability. Current focus is primarily on patient variability; in particular patient genetics. While genetics is important, it is only part of the equation. As exemplified by Case 5, therapy outcome variability might be due a combination of patient factors (e.g., genetics) and product factors (e.g., protein aggregate level at point of care). By taking both patient and product factors into account, one can better understand the causes of therapy outcome variability, which may lead to better control of outcome variability.

There is much uncertainty as to the relationship between subvisible protein aggregates and immunogenicity [53]. This is an important topic for both regulators and drug makers. Definitive answers to these questions so far have been elusive, and may vary from drug to drug [14,54]. Point-of-care QC data might help to answer questions concerning the relationship between subvisible protein aggregates and immunogenicity. Essentially, manufacturing imprecision and variable distribution history will likely lead to variable protein aggregate levels at the point of care. By comparing point-of-care data on protein aggregation with data on anti-drug antibodies in patients, one might establish the correlation, or lack thereof, between subvisible protein aggregates and immunogenicity for a given drug product.

Collecting data on every vial of biopharmaceuticals and analyzing the data trend over multiple batches can aid the consistency approach to biopharmaceutical product release, also called the 3Rs principle (Replace, Reduce and Refine animal testing) [55]. The aim of this approach is to use thorough *in vitro* characterizations to reduce the need of *in vivo* animal studies for vaccine release. This approach recognizes the need to update drug product QC and regulation with modern science [56].

Where to Start?

We anticipate the transition from inference- to verification-based QC for biopharmaceuticals will be a gradual process. Here are a few areas that, in our view, can most benefit from verification-based QC.

Originators versus follow-ons: side-by-side comparison of point-of-care data on an originator and its biosimilars might differentiate them. For example, a biosimilar might have fewer protein aggregates at the point of care than the originator has. Such comparison can be extended to nonbiological complex drugs (NBCDs), such as iron carbohydrates [57]. Whether the approval of NBCD generics should use the pathway of small-molecule generics or a separate pathway, akin to that of biosimilars, is a matter of ongoing discussion [58,59]. Point-of-care data may provide valuable input in this matter.

Strategic national stockpile (SNS): SNS contains millions of doses of biopharmaceuticals in preparation of national emergency. For example, Anthim and Raxibacumab are monoclonal antibodies in SNS for treating anthrax. Both are formulated as highly concentrated aqueous solutions and the protein aggregation level during storage might rise and vary from unit to unit in an unpredictable manner (see Case 5). Periodic quantitative inspection of biopharmaceuticals will help to ensure the readiness of the SNS.

Compounding pharmacies: biopharmaceuticals prepared or repackaged by compounding pharmacies can benefit from 100% quantitative inspection, especially when the repackaged product is used off label. It is known that off-label drug usage is associated with higher incidents of adverse drug events [60].

Vaccines: many vaccines require cold chain during distribution, but violations of cold chain are not always rare (http://oig.hhs.gov/oei/reports/oei-04-10-00430.pdf); such violations might

result in postrelease defects. Vaccines in many cases are administered to large populations, where low rate defects, say resulting from cold chain violations, may end up hurting someone. Finally, vaccines are often administered to healthy people. When serious adverse events occur, even at a low rate, and the official conclusion is, 'no evidence of causal link', the public may become distrustful of vaccination programs. The Fluad case in Italy is one example. Similar phenomena occurred in other places with other vaccines [39]. Collecting data on every vial of vaccine before vaccination may go a long way toward improving vaccine safety and, perhaps more importantly, public confidence in vaccination. This may also help to reduce animal usage in vaccine batch release testing, according to the consistency approach [55,56].

Concluding Remarks and Future Perspective

We suggest transitioning biopharmaceutical QC from inference- to verification-based practice through technology development. We envision wNMR to be one of several nondestructive quantitative inspection technologies. We also envision integration of inspection technologies with information technologies so that, after point-of-care inspection, the data are automatically recorded and transmitted to relevant parties (doctors, pharmacies, drug makers, payers, FDA, CDC, etc.). Similar to mobile phones transforming how we communicate, nondestructive inspection technologies have the potential to transform how we use drug products. To achieve this goal, the simplicity, flexibility, and affordability of the inspection technology needs to match those of mobile phones (see Outstanding Questions).

Of course, verification-based QC requires more than technical capabilities. There are also policy, legal, and financial issues to be resolved, which will likely present a greater challenge than technological issues. On the positive side, while controlling drug prices might be contentious due to the diverging interests of stakeholders, improving drug product quality is aligned with the interests of drug makers, payers, and patients, and therefore is more likely to achieve consensus. Indeed, technological advances may change long-standing practices. For example, advances in DNA sequencing technologies have led to new forensic data that were inconceivable 20 years ago but are now routine; such data reduced the chance of wrongful convictions. Likewise, data collected on every vial of a drug received by patients will likely reduce the chance of adverse drug events and save lives.

Compared with inference-based QC, verification-based QC collects more data on drug products in more diverse settings by a much wider population. This aspect facilitates the implementation of the 21st Century Cures Act, which encourages utilizing real-world evidence for drug development (https://www.congress.gov/bill/114th-congress/house-bill/34). Point-of-care data on drug product quality that is collected by patients and care providers are **real-worlddata (RWD)**.

Outstanding Questions

What are the false-positive and false-negative rates of wNMR?

Can the NMR instrument for wNMR be made small, affordable, and robust enough that nurses, pharmacists and even patients can conduct inspection on a routine basis?

Can a small wNMR device be integrated with and/or connected to smartphones?

In addition to wNMR, can nondestructive technologies based on other physical phenomena be developed?

Will 100% inspection of drug products require a change in regulation?

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Yihua Bruce Yu and Marc Taraban are listed as inventors on issued and pending patents related to wNMR.

Supplemental Information

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