PCR Unchained:

The Coming Revolution in Field-based Pathogen Detection

by

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ExcitePCR

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PREFACE

This White Paper has been written for three primary purposes:

- To provide a scientifically accurate yet basic introduction to the world of **Pathogens** — biological organisms that cause *(or lead to)* disease and/or death among humans, animals and/or plants;
- To provide in parallel a foundational history of the inventions and technologies (up to today) that are used to accurately detect and identify various pathogenic organisms; and
- To propose what we believe are 12 Factors presently limiting the widespread adoption of pathogen detection systems that utilize ***Polymerase Chain Reaction*** (aka, PCR) *In-the-Field* at what is known as at the Point-of-Care/Point-of-Need (POC/PON).

Although we, the authors, are clearly employed by an organization that is developing PCR-based pathogen detection technologies and products, we have endeavored to write this White Paper as objectively as possible to provide readers with insights into what we believe is a critically important topic.

Sincerely,

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and

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WHITE PAPER OVERVIEW

The existence of tiny organisms known as bacteria was first postulated in 1676, even though the discovery could not be affirmed by others for more than 100 years.

Advancements in science and medicine in the 1700s and 1800s led many scientists and doctors to study diseases that grew into epidemics (*a field known as* ***Epidemiology***), as many struggled to understand what was causing these clusters of suffering and death.

It's not surprising, then, that epidemiologists created a word to encapsulate the root causes behind these outbreaks: **Pathogen**. When broken down into the original Greek, the word pathogen literally means *producer of suffering (or passion).*

From a practical standpoint, pathogens attack other living organisms and take several forms, including

- Bacterium,
- Fungi,
- Prions,
- Protozoa,
- Viruses, and
- Other organisms that can cause disease and even death.

This White Paper — **PCR Unchained:** *The Coming Revolution in Field-based Pathogen Detection* — is designed to provide

1. An overview of the history of pathogen detection,
2. A review of the birth of ***Polymerase Chain Reaction***, followed by an examination of
3. The limitations and challenges of current PCR-based products and technologies.

This White Paper then closes with a brief prognostication on the future of PCR-based pathogen testing and detection systems as well as the prospect for what the authors suspect may be a "*Golden Age* of adoption and deployment of PCR-based systems" at the Point-of-Care/Point-of-Need (POC/PON).

PATHOGEN DETECTION — THE HISTORICAL PERSPECTIVE

The man most often credited with the discovery of bacteria and other single-cell organisms — **Antonie van Leeuwenhoek** — lived from 1632 to 1723. Interestingly, **van Leeuwenhoek's** role as the *Father of Microbiology* started innocently enough as an effort to make better magnifying lenses through which he could more closely examine threads in his drapery shop.

The advancements of microscopic viewing first came to light when a friend recommended **van Leeuwenhoek's** earliest invention to the Royal Society of London. Although he initially resisted the potential pull and fame of publication, **van Leeuwenhoek** eventually saw 190 of his letters published by the Royal Society over a 50-year period.

It can rightly be suggested that **van Leeuwenhoek's** magnifying lens inventions heightened the interest in exploring life that could not be seen with the naked eye. Hence, countless scientists *(both amateur and professional)* sprung forth, some with inherent curiosity as their muse, with others driven by goals of achieving fame and fortune by advancing the wealth of scientific and medical knowledge.

Yet many false beliefs dogged these efforts, such as **Aristotle's** postulate that life could spontaneously generate out of nothing. In fact, it wasn't until the work of **Louis Pasteur** in 1859 (*supported later by his friend, **John Tyndall***) that the concept of *Spontaneous Generation* was forever thrown to the dustbin of history and supplanted by the *Theory of Biogenesis* — the idea that "All Life comes from Life" (or in Latin, *Omne vivum ex vivo*).

It was the work of **Pasteur**, along with the efforts of **Robert Koch** and **Ferdinand Cohn**, that the existence of pathogenic organisms was first understood — microscopic life that could sicken, maim or kill humans and animals, or harm/destroy plant life. Among his discoveries, **Koch** is credited with uncovering the pathogens that caused such diseases/conditions as *Tuberculosis* and *Cholera*, as well as his studied observation of acquired immunity among the inhabitants of New Guinea.

Conversely, **Cohn** focused most of his career on the study of biology and plant life, with the last 28 years of his life centered primarily on bacteria — including his decision to classify bacterium into four groupings based upon cell shape. **Cohn** was also a central figure in the use of sterile culture media to grow and examine bacteria, a work improved upon by his assistant, **Julius Richard Petri**, who is credited with the invention of the *Petri Dish*.

These pioneers blazed their respective trails from the mid- to late-1800s and even into the early 1900s, work that prepared the figurative ground for the scientific endeavors of those who followed them. And yet their foundational work was unable to cross the chasm to identify the existence of a different class of pathogens: Viruses.

The number of individuals credited with discovering that disease- and death-causing organisms existed that were smaller than bacteria is too vast to describe in any detail within this White Paper. But much of their work spanned the late 1800s and the early 1900s, with notable individuals including scientists such as

- **Dimitry Ivanovsky** (credited with discovering the *Tobacco Mosaic Virus*),
- **Freidrich Loeffler** and **Paul Frosch** (credited with discovering the viral cause for *Foot and Mouth Disease*),
- **Carlos Finlay** (who identified mosquitoes as the viral carriers for *Yellow Fever*), and
- **Felix d'Herelle** (credited with the discovery of bacteriophages, viruses that infect bacterium).

CELLULAR BIOLOGY AND DNA/RNA REPLICATION

The final *a-ha moment* required to rapidly advance the field of pathogenic identification occurred at the molecular level with the discovery of DNA. The foundational molecule that carries the genetic instructions required for reproduction, growth, development and function of all organisms and many viruses is Deoxyribonucleic acid or DNA.

DNA generally exist as strands comprised of combinations of nucleobases — *Cytosine (C) and Guanine (G), and Adenine (A) and Thymine (T)* — each containing mixtures of hydrogen, oxygen, nitrogen, carbon and phosphorus atoms. The unique order and interlaced "pairing" of the C and G and the A and T nucleobases on opposite sides of a DNA molecule are what provide the genetic building blocks for most organisms. Interestingly, the two strands within a DNA molecule mirror each other, but do so in reverse (or an opposite) order.

Within cells containing a nucleus (*animals, plants, fungi and protists*), DNA strands are organized into long structures known as chromosomes that have a beginning and an end-point. Conversely, bacteria typically have DNA strands organized in a circular structure.

By contrast, viruses do not contain a nucleus, which may help explain that viral genetic material is present as either single- or double-stranded molecules of DNA or RNA (Ribonucleic acid), with the RNA molecules organized into different shapes depending upon the organism. Regardless of the shape such DNA and/or RNA molecules take, however, the reality is that growth can only occur with the separation and replication of the respective DNA / RNA molecules. DNA and RNA separation and replication occurs with the introduction of enzymes particular to the molecule in question and the particular process.

In 1956, **Arthur Kornberg** and his colleagues discovered the first identified DNA enzyme that replicated the opposing strands of DNA to create two complete DNA molecules where previously there had only been one. This first enzyme was known as DNA Polymerase (aka, Pol I) and was initially characterized to replicate *E. Coli (Escherichia coli)* in a lab environment. Since then, numerous Polymerase enzymes have been identified that produce DNA and RNA replication.

POLYMERASE CHAIN REACTION AND PATHOGEN DETECTION

Prior to 1983, the only way healthcare professionals and scientists could prove the presence of a particular pathogen was to

- Collect a sample believed to be contaminated with the suspected pathogen,
- Attempt to grow a sufficient amount of the suspected pathogen in a secure laboratory setting, typically in a culture (such as within a Petri Dish), so that
- The targeted pathogen could be accurately identified.

And although such an approach has proven to be generally successful, the labor, time and risks necessary to accurately identify targeted pathogens

1. Requires skilled healthcare and/or scientific professionals who
2. Operate in pristine environments, while
3. *Lab-grown specimens* often take several days to produce accurate results …

time not always available, especially in the midst of highly contagious pathogenic outbreaks.

Not surprisingly, many scientists looked to the discovery of polymerase enzymes as possible pathways forward to overcome the challenges presented by growing suspected pathogens in laboratory settings. Unfortunately, it was over 25 years before biochemist, **Kary Mullis**, uncovered the key to rapidly and accurately replicate DNA strands over and over again while working for Cetus Corporation, a process he called ***Polymerase Chain Reaction*** (or PCR for short).

Numerous scientists and organizations have since built upon Mullis' original PCR discovery, including corporations such as Hoffham-La Roche (*which acquired Cetus' PCR-related patents and intellectual properties in 1991 for $300 million*). Today, all of the original Cetus derived patents have expired, while in-lab usage of PCR-based systems has been widely adopted around the globe.

Yet as pervasive as PCR systems are in controlled laboratory settings, their usage *In-the-Field* in what is typically described as at the Point-of-Care/Point-of-Need dramatically lags behind lab usage.

Why this is the case and the steps that must be taken to further benefit society from rapid and accurate POC/PON PCR-based pathogen detection serve as the balance of this White Paper.

12 FACTORS CURRENTLY LIMITING GREATER ADOPTION OF PCR-BASED SYSTEMS AT THE POINT-OF-CARE/POINT-OF-NEED

In the 30+ years since **Mullis** revealed the path forward via ***Polymerase Chain Reaction***, it is clear that the adoption of PCR-based pathogen detection has spread around the globe. In fact, worldwide revenue projections for PCR systems are pegged to reach over $28 billion (US) within the next five years.

Clearly, PCR-driven systems provide a significant advantage over traditional, lab-based pathogen identification and detection protocols where healthcare and medical professionals grow specimens in pristine laboratory settings. Yet we wonder if PCR adoption has, in fact, actually plateaued or perhaps paused? And if so, the question arises as to why this might be the case.

This White Paper attempts to identify what we believe are the 12 Factors most likely currently prohibiting or holding back even greater acceptance/adoption of PCR-based pathogen detection solutions. A list of these 12 Limiting Factors (outlined in reverse order) is found below. *{NOTE: We do **not** see these 12 Factors as permanent challenges; rather, we believe them to be temporary in nature.}*

12. Disposal/Storage Concerns in POC/PON Settings

The Twelfth Most Pressing Issue we see currently limiting POC/PON-based PCR deployment concerns related storage and disposal matters. Specifically, as challenging as the logistics can be with Point-of-Care/Point-of-Need pathogen testing and detection, another less obvious difficulty in a field-based POC/PON setting can be post-test storage and/or disposal of PCR-related materials. This can be especially true in a remote or rural environment where the presence of highly contagious organisms is suspected.

11. Resupply Issues in POC/PON Settings

We believe the Eleventh Greatest Factor currently restricting in-field PCR adoption centers around challenges of "Resupply." Clearly, the more remote a location where a potential outbreak occurs, the more difficult ongoing support becomes the longer POC/PON testing and detection is required. The logistics behind such field-based support crosses all disciplines in a POC/PON environment, but certainly includes the timely resupply of supplies and materials to continue testing/detection.

10. Requirement of Trained Professionals to Operate PCR-based Systems

As Point-of-Care/Point-of-Need pathogen detection demands have arisen, so has the need for trained professionals to operate PCR-based systems. We feel that this conundrum is the Tenth Factor presently restraining POC/PON adoption of PCR-based solutions.

9. Accuracy Concerns for POC/PON- vs. Lab-based-PCR Testing Data

Can field-based PCR be as accurate as PCR testing and detection processes conducted in a pristine laboratory setting? We recognize that this is a foundational question for many healthcare professionals, first responders, and food safety officials. As such, we think this issue serves as the Ninth Most Pressing Factor inhibiting the proliferation of PCR systems in Point-of-Care/Point-of-Need environments today.

8. Security of Gathered Test Results

Presuming that accurate PCR-derived test results can be obtained *In-the-Field*, it is our belief that the security of such results and the samples that proffered them serve as the Eighth Factor holding back POC/PON deployment of PCR-based solutions today. We feel that such legitimate security concerns range from Data Encryption to Theft Prevention.

7. Challenges with Aggregating Data from POC/PON Settings

It is one thing to successfully identify *In-the-Field* that one victim has been infected by a virulent bio-threat. However, the risk of being unable to aggregate test results across scores or thousands of potential victims to derive critical data regarding a potential outbreak is even more concerning. We see this challenge as the Seventh Greatest Concern that has restricted PCR-based deployments in remote/rural settings thus far. *{NOTE: To be clear, such concerns also apply to animal and plant species as well.}*

6. Trained Professionals Needed to Interpret POC/PON-gathered Data

In addition to the need to have trained professionals to operate PCR-based systems today *(see No. 10 above)*, we recognize that there is often a similar need to have trained medical/scientific professionals to understand and evaluate the test results obtained via PCR-based testing and detection. We believe this reality has served as the Sixth Factor restricting the deployment of POC/PON PCR solutions to date.

5. Inadequate Communications Infrastructure in POC/PON Settings

The more remote the setting for field-based pathogen testing and detection, the more *off-the-grid* one will be, which presents a number of communication limitations for both those deployed in the field as well as those deployed "back home." In our opinion, then, communication restrictions serve as the Fifth Factor currently curbing widespread adoption of PCR-based pathogen detection in POC/PON environments.

4. Lack of Power (Electricity) in POC/PON Settings

Clearly, if communication is going to be a challenge in a remote setting, the lack of electrical power *in-the-field* will be similarly challenging. Ideally, PCR solutions deployed at the Point-of-Care/Point-of-Need would also have battery-powered systems to deliver near round-the-clock testing/detection capabilities, something not generally available today. That is why we have identified this present lack of electricity/power as the Fourth Most Crucial Factor restricting adoption of PCR solutions in POC/PON settings.

3. Size of the PCR-based Machines

The vast majority of PCR systems available today (let alone 10—20 years ago), tend to be bulky and/or heavy machines more suited for bench-top or desktop deployment in laboratory settings. We think the larger size and heavy weight of most PCR systems available today has served as the Third Worst Factor restraining widespread POC/PON adoption of PCR-based solutions.

2. Difficulty of Field-based Sample Preparation

One of the incremental advancements seen in the field of pathogen detection using ***Polymerase Chain Reaction*** systems is the ability to deliver highly accurate results in a relatively short period of time (*e.g., 60 minutes or less*). Unfortunately, comparable advancements in sample preparation before the pathogen detection process begins have not kept pace with the noted time reductions with the latest PCR systems.

Additionally, the increased possibility of contamination in a remote/rural hot-site can dramatically boost the time needed for sample preparation time prior to initiating a PCR-based detection procedure. We feel the combination of these two challenges make field-based sample preparation the Second Greatest Factor presently restricting the widespread POC/PON adoption of PCR solutions.

1. Expense

Our research of the currently available solutions suggests that today's No. 1 Factor inhibiting the use of PCR-based pathogen detection systems *In-the-Field* is **Expense**:

- The expense of *the PCR-based machines themselves*,
- The expense of *the mechanisms used by these PCR-based machines* (e.g., cartridges, pouches, assays, supplies, etc.)
- The expense of *transporting PCR-based machines* to/from remote settings (especially given the general size and weight of most generally used systems today),
- The expense of *deploying and supporting trained professionals* to, from and in remote settings, and
- The expense of *providing/augmenting electrical infrastructure* at the Point-of-Care/Point-of-Need to power PCR-based solutions.

Taken in concert, Expense is clearly the leading restrictor currently holding back the utilization of PCR-based systems in remote, in-field settings.

THE FUTURE OF *IN-FIELD* DEPLOYMENT OF PCR-BASED SYSTEMS

In our estimation, we do not believe there is one *"Magic Bullet"* that will transform the application of ***Polymerase Chain Reaction*** testing and detection solutions *In-the-Field* to create massive POC/PON adoption… especially not when we have identified what we see as 12 Factors presently restricting such usage. Please note, however, that we also believe that such *"limitations"* are not permanent, but rather are temporary in nature, especially given the ongoing pace of rapid technological advancements. And we are quite aware of several such technological advancements.

It is also clear to us that the demand for such Point-of-Care/Point-of-Need pathogen testing/detection is not going to subside. If anything, we soberly expect a dramatic increase for such *In-Field* pathogen detection in the years ahead, primarily due to natural causes, but also likely to human causes.

Hence, if/when one or more companies can produce a PCR-based pathogen testing/detection system that eliminates half or more of the Limiting Factors outlined in this White Paper, we anticipate such firms will find outsize success in the POC/PON marketplace, as well as in the wider PCR-based systems market.

In fact, we suspect that such advancements will give birth to a *Golden Age* of adoption and deployment of PCR-based pathogen testing and detection systems on a worldwide basis, an era that will greatly outstrip previous usage. We also think that such a *Golden Age* will also lead to safer societies, healthier human/animal/plant populations, and a more secure food supply.

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ABOUT ExcitePCR

ExcitePCR Corporation is developing portable realtime pathogen detection systems based upon superior *PCR (Polymerase Chain Reaction)* methodologies. Its predecessor company (*Microfluidic Systems*) was one of seven companies awarded contracts by the U.S. Department of Homeland Security in 2004 to develop a solution to automatically detect airborne pathogens. Since then, ***ExcitePCR*** has continued to advance its pathogenic testing and detection systems, and its **FireflyDX™** technologies are designed to deliver automated Point-of-Care/Point-of-Need (POC/PON) sample preparation and highly accurate biohazard identification results significantly faster than using existing *PCR*-based solutions. The company's first product is slated for commercial availability in Summer 2018.

For more information about ExcitePCR, please visit its Website at www.excitepcr.com.

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