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COVID-19 Test Kits Reagents and Formulae

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ABSTRACT

The Defense Systems Information Analysis Center (DSIAC) was tasked with researching the chemical reagents and formula of seven different SARS-CoV-2 test kits approved by the U.S. Food and Drug Administration (FDA) as of April 2020. DSIAC, with the help of a Department of Homeland Security researcher and two Homeland Defense & Security Information Analysis Center analysts, summarized the two types of COVID-19 tests (antigen and antibody) and the various reagents and chemical materials required for each test. Sources for the chemical reagents and other processes that utilize these chemicals are also discussed. Even with the increased demand for these specific chemical reagents, suppliers appear to not have significant issues involving their supply chains, and researchers are developing new tests that use different reagents. With the implementation of Emergency Use Authorization from the FDA, result speeds and new test types in the COVID-19 test kits have improved in the last few months.

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1.0 TI Request

1.1 INQUIRY

What are the reagents types and formulae used in COVID-19 test kits, sources of the reagents, and other processes using those reagents?

1.2 DESCRIPTION

The inquirer was provided a list of test kits that included the following manufacturers: Becton, Dickinson and Company (BD), Abbott, ThermoFisher, Roche, Siemens Fast Track Diagnostics, and RUCDR Infinite Biologics.

2.0 TI Response

This report includes findings based on research conducted in April 2020. In this report, the Defense Systems Information Analysis Center (DSIAC) summarizes each SARS-CoV-2 test kit, identifies chemical reagents and other ingredients that make up each kit, when available, and suggests other processes that may use those ingredients. To complete this, DSIAC subject matter expert Krysalyn Gerhardt, a forensic analytical reach-back chemist from the U.S. Customs and Border Protection (CBP) branch of the Department of Homeland Security (DHS), assisted in collecting and synthesizing the information. The Homeland Defense and Security Information Analysis Center (HDIAC) analysts Amber Garvey and Kayasha Freeman also assisted in completing this report.

2.1 REAGENTS AND FORMULAE USED

There are currently two types of tests available for SARS-CoV-2: antigen and antibody.

Antigen tests, also called molecular tests or nucleic acid amplification tests (NAAT tests), detect the virus's genetic material (DNA or RNA) and can be used by itself to diagnose COVID-19 [1]. The most commonly used SARS-CoV-2 test is a polymerase chain reaction (PCR) test in which the objective is to isolate and amplify trace amounts of genetic material—in this case, viral genetic material. A PCR test requires DNA; however, SARS-CoV-2 tests use RNA. Thus, an enzyme is required to convert RNA to DNA—namely, reverse transcriptase. This converts the polymerase chain reaction (PCR) into a reverse transcription polymerase chain reaction (RT-PCR), which is used to make a complementary DNA/RNA hybrid [2]. These molecules are manufactured in laboratories to a certain level of quality control to ensure accurate results [3].

PCR-based SARS-CoV-2 tests rely on six components—DNA, enzymes, primers, probes, nucleotides, and buffers. They are summarized as follows:

- Currently, the patient's DNA is typically collected through nasopharyngeal swab, and the DNA polymerase (DNAP) enzyme is used to separate the DNA strands.
- Primers, which are short strands of nucleotides complementary to the DNA template, initiate DNA synthesis. These primers serve as the starting point for the DNA and RNA polymerase enzymes by demarcating the section of DNA to be amplified and attaching to the location by hydrogen bonding.
- Probes function to increase specificity of the quantitative PCR.
- Nucleotides, or dNTPs (deoxynucleotide triphosphates), are the building blocks of nucleic acids and added by DNAP to the growing DNA strand during the extension step.
- Buffers are used to chemically neutralize the pH-sensitive environment, ensuring proper DNA synthesis function of DNAP [2].

Since it is particularly relevant to current test processes, the method for converting RNA to DNA is summarized as follows: reverse transcriptase is used to make complementary DNA to the viral RNA strand. This results in a DNA/RNA hybrid. The enzyme RNA polymerase then breaks up the original RNA fragment, and the remaining complementary DNA strand is duplicated. The double-stranded DNA is then ready to be amplified [4].

At the start of amplification, heat and enzymes force apart the converted, double-stranded DNA generated from the viral RNA. Once cooled, short primers, which match the complementary strand of template DNA, stick to the converted strand and create an artificial site for synthesis. New DNA strands are assembled by DNA polymerase on each of the separated strands. The probes release from the strands and fluoresce, allowing for the reading of results [2]. After the first complete round of synthesis, the reaction mixture is heated to separate the strands again. Once these strands are separated, the process starts over, amplifying viral DNA at an exponential rate. If SARS-CoV-2 is evident in the sample, the primers copy that targeted region. As copies are made, probes stick to them and release the visual fluorescent signals to communicate that SARS-COV-2 is present. If the virus is not present, the probes do not stick, there is no signal, and the test results are read as negative [5].

Antibody, or serology, tests detect antibodies in the blood when the body is fighting an infection instead of the actual virus. In the early days of an infection, antibodies may not be detected, limiting the effectiveness of an antibody test. This type of test may also be falsely positive if antibodies to a coronavirus other than the pandemic novel strain are present. Because of this, the U.S. Food and Drug Administration (FDA) has stated that antibody tests should not be used alone to diagnose COVID-19 [1]. Antibody test kits typically include a lancet to take the blood sample, a chemical buffer to help the blood flow, and a test strip that contains antibodies that will bind to specific biomarkers associated with the virus—immunoglobulin G (IgG) and immunoglobulin M (IgM). Lines will appear if a patient has

previously contracted the virus, though only one biomarker needs to be present to indicate a previous infection [3].

In this section, a short summary of each SARS-CoV-2 test kit and the reagents used in the kit is provided. However, some of the kits have limited listings or are patented mixtures, which do not provide specifics. Most kits, though, are either PCR or master mix (MMX) kits and would include something similar to the following [6]:

- PCR (could apply to Siemens, Abbott Laboratories IgG Assay, Thermo Fisher, Roche, or RUCDR)
 - Template DNA
 - Primers: oligo (dT 18) primer
 - Nucleotides: dNTPs
 - Buffer: Tris-HCl (Tris base and HCl), potassium chloride (KCl), magnesium chloride (MgCl₂)
- Master Mix (could apply to Siemens, Abbott Laboratories IgG Assay, Thermo Fisher, Roche, or RUCDR)
 - Taq DNA polymerase
 - dNTPs: dATP, dCTP, dGTP, and dTTP (substituted for dUTP with use of uracil-DNA glycosylase)
 - MgCl₂

2.1.1 Becton, Dickinson, and Company (BD) MAX Test

2.1.1.1 Introduction

BD and BioMedomics released a new point-of-care (POC) test that can detect antibodies in blood to confirm current or past exposure to COVID-19 in as little as 15 min. This test does not require special equipment and can be used in a laboratory or at the POC. The test detects antibodies in the blood that are produced by the body in response to coronavirus infection. These antibodies are typically present in the middle-to-late stages of the infection, though may remain after exposure, which helps clinicians determine the exposure of both symptomatic and asymptomatic persons. The test is completed in four steps: (1) blood is collected through normal measures; (2) a few drops of blood are transferred to the test cartridge; (3) two or three drops of buffer are added; and (4) the results can be read in 15 min.

The test analyzes blood, serum, or plasma samples for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies associated with the coronavirus (SARS-CoV-2 or COVID-19). IgM provides the first line of defense during viral infections, followed by the generation of adaptive, high-affinity IgG responses for long-term immunity and immunological memory. The detection of IgM antibodies indicates a more recent exposure to COVID-19, while the appearance of IgG antibodies indicates a later stage of the infection. The FDA recommends

that the results from antibody testing should not be used as the sole basis to diagnose or exclude the coronavirus.

This test, developed and manufactured by BioMedomics, will be distributed exclusively by Henry Schein, Inc. to U.S. health care providers. BD expects to be able to supply more than a million tests in the coming months of 2020, with the ability to scale up production based on market demand [7].

2.1.1.2 Reagents and Formula

This test detects antibodies IgM and IgG associated with COVID-19 and determines if someone has had the virus. It uses the BD Microtainer Contact-Activated Lancet, which transports the blood into a buffer [7]. While not being able to determine the precise buffer used, according to the FDA, it could be a phosphate buffer containing sodium chloride (NaCl), ethylenediaminetetraacetic acid (EDTA), commercially available detergent nonyl phenoxyethoxyethanol (NP-40), polysorbate-type nonionic surfactant polysorbate 20 (Tween 20), and chicken serum, urea, antimicrobials, and sodium azide as a preservative [6, 8].

2.1.2 Abbott Laboratories SARS-CoV-2 IgG Assay

2.1.2.1 Introduction

Abbot Laboratories launched a laboratory-based blood test designed to detect antibodies for coronavirus detection. The test is performed using the SARS-CoV-2 IgG Reagent Kit in combination with the SARS-CoV-2 IgG Calibrator Kit on the Architect i1000SR and i2000SR laboratory instruments [9, 10]. The SARS-CoV-2 IgG Assay is designed to detect immunoglobulin class G (IgG) antibodies to the nucleocapsid protein of SARS-CoV-2 in serum and plasma (ACD, CPD, CPDA-1, dipotassium EDTA, tripotassium EDTA, lithium heparin, lithium heparin separator tube, sodium citrate, and sodium heparin) from individuals suspected to have had coronavirus disease (COVID-19) or in serum and plasma of subjects that may have been infected by SARS-CoV-2. This assay is an automated, two-step immunoassay for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma using chemiluminescent microparticle immunoassay technology. The presence or absence of IgG antibodies to SARS-CoV-2 in the sample is determined by comparing the chemiluminescent relative light units (RLU) in the reaction to the calibrator RLU [10].

Abbott Laboratories has shipped out nearly 2.5 million tests to customers across the United States and plans to ramp up to 20 million antibody tests in June and beyond. They also plan to expand the testing capabilities to their Alinity i lab system [9].

2.1.2.2 Reagents and Formula

The Abbott Laboratories SARS-CoV-2 IgG Reagent Kit 06R86 includes the following reagents [10]:

- **M:** Purified SARS-CoV-2 recombinant antigen coated microparticles in TRIS buffer with surfactant. Minimum concentration: 0.045% solids. Preservatives: ProClin 950 and sodium azide.
- **Conjugate:** Antihuman IgG (mouse and monoclonal) acridinium-labeled conjugate in 2-morpholinoethanesulfonic acid buffer with surfactant and protein (bovine) stabilizer. Minimum concentration: 4 ng/mL. Preservatives: ProClin 300 and antimicrobial agents.
- **Assay:** Tris buffer and detergent. Preservatives: ProClin 950 and sodium azide.

2.1.3 Abbott Laboratories ID NOW COVID-19 Test

2.1.3.1 Introduction

Abbott received emergency use authorization (EUA) from the FDA for the fastest available POC test for detecting the COVID-19, delivering positive results in as little as 5 min and negative results in 13 min [11]. This antigen test is an automated assay that utilizes isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral nucleic acids [12]. Abbott's ID NOW COVID-19 test can be used outside traditional hospitals, as the test runs on Abbott's ID NOW platform – a lightweight box (6.6 lb and the size of a small toaster) that can be used in nontraditional places. Abbott was preparing to increase production to deliver 50,000 ID NOW COVID-19 tests per day beginning in early April [11].

2.1.3.2 Reagents and Formula

The Abbott Laboratories ID NOW COVID-19 test is comprised of a sample receiver (containing elution/lysis buffer), a test base (comprising two sealed reaction tubes, each containing a lyophilized pellet), a transfer cartridge for transferring the eluted sample to the test base, and the ID NOW instrument [12]. This means the test includes lyophilized reagents (a mixture of a matrix [caking agent] and lyoprotectant that act synergistically to provide a solution with a high eutectic point, solid matrix, and excellent protein stability) and an elution buffer (Tris-HCl and EDTA). Lyophilized reagents could include dNTPs and NTPs, DTT (dithiothreitol), ITP (inosine-5'-triphosphate, sodium salt), KCl, and MgCl₂ [6].

2.1.4 ThermoFisher Applied Biosystems TaqPath COVID-19 Combo Kit

2.1.4.1 Introduction

ThermoFisher has developed a new multiplex, real-time RT-PCR diagnostic kit to enable clinical and public health laboratories to diagnose COVID-19 infections that is being released under EUA. The TaqPath COVID-19 Combo Kit is a fast and highly sensitive multiplex diagnostic solution that contains both the assays and controls need for real-time detection of RNA from the SARS-CoV-2 virus on the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument. The kit can be used to quickly evaluate up to 94 patient specimens in under 3 hr. This antigen test is approved for use with RNA extracted from nasopharyngeal swabs, nasopharyngeal aspirate

(nasal aspirate), and bronchoalveolar lavage from patients at risk of exposure to SARS-CoV-2 or with signs and symptoms of COVID-19 [13, 14].

2.1.4.2 Reagents and Formula

The Applied Biosystems TaqPath RT-PCR COVID-19 Kit contains the Applied Biosystems TaqPath COVID-19 Assay Multiplex reagent (assays and controls), the three primer/probe sets specific to different SARS-CoV-2 genomic regions (Gene Orf-1ab, N protein, S protein) and primers/probes for bacteriophage MS2, and the MS2 Phage Control reagent. The TaqPath COVID-19 Control Kit includes an RNA positive control that contains the SAR-CoV-2 genomic regions targeted by the kit and the TaqPath COVID-19 Control Dilution Buffer [6, 14].

Another RT-PCR test, which includes SYBR Green PCR MMX combines SYBR Green I dye, AmpliTaq Gold DNA polymerase, dNTPs with dUTP, Passive Reference dye ROX, and optimized buffer in the convenience of a single vial. This test utilizes a nucleic acid isolation kit that requires a lysis buffer. Most lysis buffers contain buffering salts and ionic salts to regulate the pH and osmolarity of the lysate. An example would include Tris-HCL buffer, EDTA, and sodium dodecyl sulfate (SDS) [6, 13].

2.1.5 Roche cobas SARS-CoV-2 Test

2.1.5.1 Introduction

Roche's cobas SARS-CoV-2 test is an antigen test that was issued an EUA in mid-March. The cobas SARS-CoV-2 test is a single-well, dual-target assay that includes both the specific detection of SARS-CoV-2 and pan-sarbecovirus detection for the sarbecovirus subgenus family, which includes SARS-CoV-2. The test is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in nasopharyngeal and oropharyngeal swab samples collected in Copan Universal Transport Medium Systems or BD Universal Viral Transport System and analyzed using Roche's fully automated cobas 6800 or 8800 systems. This process can provide 96 patient results in roughly 3 hours and a total of 384 results for the cobas 6800 system and 960 results for the cobas 8800 system in 8 hours [15, 16].

Roche is closely monitoring how the pandemic affects their overall supply due to the assumption that demand will outstrip supply at the height of the pandemic. Their manufacturing network has a global footprint, with plants located in China (does not supply to the United States), Switzerland, Germany, South Africa, and the United States. In a March 11 memo, Roche had not experienced any supply disruptions for the raw materials [17].

2.1.5.2 Reagents and Formula

The Roche cobas SARS-CoV-2 test includes the reagents and controls listed in Tables 1–3 [15].

Table 1: Roche cobas SARS-CoV-2 Test Kit

Kit Components	Reagent Ingredients	Quantity per Kit
Proteinase Solution (PASE)	Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase	22.3 mL
RNA Internal Control (IC)	Tris buffer, <0.05% EDTA, <0.001% nonsarbecovirus-related, armored RNA construct containing primer and probe-specific primer sequence regions (noninfectious RNA in MS2 bacteriophage), <0.1% sodium azide	21.2 mL
Mastermix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, <0.1% sodium azide	7.5 mL
SARS-CoV-2 Master Mix Reagent 2 (SARS-CoV-2 MMX-R2)	Tricine buffer, potassium acetate, <18% dimethyl sulfoxide, glycerol, <0.1% Tween 20, EDTA, <0.12% dATP, dCTP, dGTP, dUTPs, <0.01% internal control forward and reverse primers, <0.01% fluorescent-labeled oligonucleotide probes specific for SARS-CoV-2, sarbecovirus, and the RNA internal control, <0.01% oligonucleotide aptamer, <0.1% Z05D DNA polymerase, <0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial), <0.1% sodium azide	9.7 mL

Table 2: cobas SARS-CoV-2 Control Kit Ingredients

Kit Components	Reagent Ingredients	Quantity per Kit
SARS-CoV-2 Positive Control (SARS-CoV-2 (+)C)	Tris buffer, <0.05% sodium azide, <0.005% EDTA, <0.003% Poly rA, <0.01% noninfectious pasmid DNA (microbial) containing SARS-CoV-2 sequence, <0.01% noninfectious plasmid DNA (microbial) containing pan-sarbecovirus 1 sequence, <0.01% noninfectious plasmid DNA (microbial) containing pan-sarbecovirus sequence	16 mL

Table 3: cobas omni Reagents for Sample Preparation

Reagents	Reagent Ingredients	Quantity per Kit
cobas omni MGP Reagent (MGP)	Magnetic glass particles, Tris buffer, 0.1% methyl-4-hydroxybenzoate, <0.1% sodium azide	480 tests
cobas omni Specimen Diluent (SPEC DIL)	Tris buffer, 0.1% methyl-4-hydroxybenzoate, <0.1% sodium azide	4 x 875 mL
cobas omni Lysis Reagent (LYS)	43% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	4 x 875 mL
cobas omni Wash Reagent (WASH)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L

2.1.6 Siemens Fast Track Diagnostics SARS-CoV-2 Assay

2.1.6.1 Introduction

Siemens developed the FTD SARS-CoV-2 Assay as a real-time PCR test to detect the novel coronavirus by targeting ORF1ab and N gene regions in virus RNA. The FTD SARS-CoV-2 Assay uses the same protocol, including PCR cycling profile, as all fast-track diagnostics (FTD) respiratory assays. A Siemens FTD Kit includes a mastermix, positive and negative controls, and IC [18].

2.1.6.2 Reagents and Formula

The Siemens FTD SARS-CoV-2 Assay (FTD-114-32-RUO or SMN-11416314) includes the following components: primer/probe mix, enzyme and buffer, internal control (equine artiritis virus), and positive and negative controls [18]. NucliSENS easyMAG (bioMeriueX) for extraction, which is comprised of silica, lysis buffer (buffering salts and ionic salts, e.g., Tris-HCl buffer, EDTA, and SDS), wash buffers (sodium acetate and ethanol or isopropanol) [6, 19].

2.1.7 RUCDR Infinite Biologics

2.1.7.1 Introduction

The test developed by RUCDR Infinite Biologics, a biorepository backed by Rutgers University, is an outlier; it features saliva testing and is currently the only emergency use authorization (EUA) SARS-CoV-2 diagnostic test that does so. It builds upon the existing TaqPath SARS-CoV-2 Assay used in other COVID-19 testing. On March 8, 2020, the FDA issued an EUA to allow these saliva tests to be used on an at-home basis [20, 21].

Saliva collection is less invasive than nasopharyngeal testing and requires fewer resources, personal protective equipment, and trained personnel. Further, between SARS-CoV-2

detection from nasopharyngeal and saliva samples, it was found that saliva yielded higher detection sensitivity and less variability in self-sample collection. These findings suggest that saliva is a viable and more sensitive alternative to nasopharyngeal swabs and could facilitate at-home, self-administered sample collection for accurate large-scale SARS-CoV-2 testing [21, 22]. The test is a real-time reverse transcription polymerase chain reaction test that uses primers and probes to identify RNA from the virus in respiratory specimens from the patient. RNA extraction for all specimen types is performed using the PerkinElmer Chemagic 360 automated specimen processing system with the Chemagic Viral DNA/RNA 300 Kit H96. The input sample volume is 300 μ L, and the elution volume is 50 μ L. Reverse transcriptase-PCR (RT-PCR) is performed using the Applied Biosystems TaqPath COVID-19 Combo Kit with 5 μ L of the extracted sample [23].

RUCDR’s current capacity for saliva tests is 10,000 samples per day and is expected to increase to between 30,000 to 40,000 samples per day in the coming weeks [24].

2.1.7.2 Reagents and Formula

The RUCDR test requires the use of a saliva collection device from Spectrum Solutions, which contains patented preservation reagents to stabilize RNA and DNA [24]. According to the FDA’s EUA document, the reagents and materials required from the using this test are listed in Table 4 [23].

Table 4: Reagents and Materials Required for the RUCDR Saliva-Based COVID-19 Test

Reagent	Manufacturer	Catalogue #
Chemagic Viral DNA/RNA 300 Kit H96	PerkinElmer	CMG-1033-S
96 Well, Deep Well Plates	PerkinElmer	43001-0120
TaqPath COVID-19 Combo Kit	ThermoFisher Scientific	A147814
384 Well PCR Plate	ThermoFisher Scientific	4483273
Optical Adhesive PCR Plate Cover	ThermoFisher Scientific	4311971
Nuclease-Free Water	—	—
Ethanol (96–100%)	—	—

2.2 REAGENT SOURCES

Public health laboratories can access collection materials for SARS-CoV-2 testing, including swabs and transport media, through the International Reagent Resource (IRR). The IRR supports qualified laboratories by providing reagents, tools, and information for studying SARS-CoV-2 and other pathogens free of charge [25]. The IRR acquires, authenticates, and produces reagents that scientists need to carry out basic research and develop improved diagnostic tests, vaccines, and detection methods [26].

Clinical and commercial laboratories conducting SARS-CoV-2 viral testing can acquire test reagents from commercial reagent manufacturers who have received EUA from the FDA [25]. One such commercial reagent manufacturer/retailer is Sigma-Aldrich, or MilliporeSigma. This includes buffer solutions, such as Tris-HCl (or separately, Tris base and HCl), KCl, MgCl₂, lysis buffer ingredients (buffering salts and ionic salts: Tris-HCl buffer, EDTA, and SDS), wash buffers (sodium acetate, ethanol, isopropanol), phosphate buffer (or components NaCl, EDTA, NP-40, Tween 20, chicken serum, urea, antimicrobials, and sodium azide), dimethyl sulfoxide, and bovine serum albumin (BSA). They also sell SYBR Green PCR Master Mix, as well as the separate components, including the necessary primers, nucleotides, and Taq polymerase [6, 27]. Other chemical suppliers include Merck, ThermoFisher, and Agilent [28].

Though the aforementioned tests require the same various reagents, a new testing method that is “500 times more sensitive than the standard coronavirus test” adapted and validated by Michigan State University (MSU) does not. Bio-Rad Laboratories has developed and manufactured a machine that MSU used to validate their test. This test is based off information posted by Chinese researchers, which used different chemical reagents [29].

While it might seem like demand is outstripping potential supply of tests and/or the chemical reagents involved, it does not appear to be the case as supply companies like Merck & Co. report minimal impact on their supply chain for raw materials. Therefore, enzyme supply does not appear to be an issue, though the supply of buffers used for RNA extraction could be strained since they are typically proprietary systems. Regardless, the Chemical Industries Association stated that global industries are working to meet the increased demands for the materials required to test for SARS-CoV-2. It may turn out that the shortages in the supply chain do not involve reagents but instead involve the required instruments, trained technicians, and non-qPCR assays [30].

2.3 OTHER PROCESSES USING REAGENTS

Many of the following reagents have typical commercial use in biology. Schools may use some of these reagents for laboratory experiments that might not be held during the pandemic.

PCR is a common step in DNA analysis. DNA testing companies such as 23andMe, Ancestry, and MyHeritage DNA might have these supplies on hand, which could be freed up in this time, as genealogical testing is not essential. Furthermore, PCR is used in research and practical applications such as DNA cloning, medical diagnostics, and forensic analysis of DNA.

Magnesium chloride (MgCl₂) is an important compound with various uses in the food industry, health, road maintenance, and industrial applications. Its high solubility in water makes it perfect for waste-water treatment, feed supplements for livestock, road stabilization, fireproofing agents, etc.

Potassium chloride (KCl) is used for the manufacture of potassium hydroxide and potassium metal. It is also used in medicine, lethal injections, scientific applications, food processing, soaps, and as a sodium-free substitute for table salt for people concerned about the health effects of sodium. Potassium chloride is used in the manufacture of potash, an important form of fertilizer that enriches soils with potassium, which promotes the growth of plant life. The use of potassium chloride as a source of beta radiation is extremely useful in calibrating radiation monitoring equipment.

Edetate disodium (EDTA) is a chelating agent. A chelating agent can remove a heavy metal, such as lead or mercury, from the blood. EDTA is used to lower blood levels of calcium when they have become dangerously high. In manufacturing, EDTA is used to improve stability of some pharmaceutical products, detergents, liquid soaps, shampoos, agricultural chemical sprays, contact lens cleaners, and cosmetics. It is also used in certain blood collection tubes used by medical laboratories.

Sodium lauryl sulfate, referred to as **sodium dodecyl sulfate (SDS)** in science, is used in cleaning and beauty products. SLS is known as a “surfactant.” This means it lowers the surface tension between ingredients, which is why it is used as a cleansing and foaming agent. It is used in grooming products, such as shaving cream, lip balm, hand sanitizer, nail treatments, makeup remover, foundation, facial cleansers, exfoliates, liquid hand soap, hair products (such as shampoo, conditioner, hair dye, dandruff treatment, and styling gel), dental care products (such as toothpaste, teeth whitening products, and mouthwash), bath products (such as bath oils or salts, body wash, and bubble bath), and creams and lotions (such as hand cream, masks, anti-itch creams, hair-removal products, and sunscreen). SLS is also used as a food additive, usually as an emulsifier or a thickener. It can be found in dried egg products, some marshmallow products, and certain dry beverage bases.

NP-40 is often used to break open all membranes within a cell, including the nuclear membrane. To break only the cytoplasmic membrane, other detergents such as IGEPAL CA-630 can be used. NP-40 has applications in paper and textile processing, in paints and coatings, and in agrochemical manufacturing.

Tween 20 (Polysorbate 20) is a common component in the following applications: the pre-extraction of membranes to remove peripheral proteins, membrane-based immunoassays, lysing mammalian cells, PCR, ELISA (enzyme-linked immunosorbent assay), and western blot analysis. Polysorbate 20 is used by philatelists to remove stamps from envelopes and residues from stamps, without harming the stamp itself. It is also used as wetting agent in rubber balers in the elastomer industry. It has also been used as a shape-directing agent to synthesize spheroidal magnetite nanoassemblies.

Chicken serum is a nutrient-rich fluid derived from the blood of healthy chickens but does not contain red blood cells or other clotting components. The composition of chicken serum allows it to be used in biological and chemical research applications.

Urea can be manufactured from a combination of phosgene and ammonia. Most urea produced is used as a fertilizer. Its other uses include the manufacture of the melamine, used in melamine-methanal resins. Urea itself also forms important resins. An increasingly important use of urea is in reducing air pollution from diesel engines in cars, buses, and lorries. Urea is a raw material used in the manufacture of many chemicals, such as various plastics, urea-formaldehyde resins, and adhesives. It is also essential for making feedstock, glue, fertilizer, and commercial products.

Sodium azide is used as a chemical preservative in hospitals and laboratories. It is also used in agriculture for pest control and sometimes in detonators and other explosives.

Dimethyl sulfoxide (DMSO) is used topically to decrease pain and speed the healing of wounds, burns, and muscle and skeletal injuries. DMSO is also used topically to treat painful conditions such as headache, inflammation, osteoarthritis, rheumatoid arthritis, and severe facial pain (called tic douloureux). DMSO industrial grade is used as a reaction solvent in manufacturing a variety of polymers. Additional downstream applications include a processing solvent in operations, such as casting polymeric membranes and wet-spinning synthetic fibers.

Bovine serum albumin (BSA) is used for stabilization of enzymes during storage and for enzymatic reactions where the absence of nucleases is essential. BSA increases PCR yields from low-purity templates and prevents adhesion of enzymes to the reaction tubes and tip surfaces. Bovine serum albumin is found in milk and in whey proteins in varying amounts.

Taq polymerase is a thermostable DNA polymerase named after the thermophilic eubacterial microorganism *Thermus aquaticus*, from which it was originally isolated by Chien et al. in 1976 [31]. Its name is often abbreviated to "Taq" or "Taq pol." It is frequently used in the PCR, a method for greatly amplifying the quantity of short segments of DNA. *T. aquaticus* is a bacterium that lives in hot springs and hydrothermal vents, and Taq polymerase was identified as an enzyme able to withstand the protein-denaturing conditions (high temperature) required during PCR.

MS2 bacteriophage can be used as an internal control in nucleic acid-based amplification assays to monitor reverse-transcription and PCR inhibition. Controls should be run using the same protocols as those used to amplify extracted clinical specimens [6].

REFERENCES

- [1] FDA. "Coronavirus Disease 2019 (COVID-19) Frequently Asked Questions." <https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/coronavirus-disease-2019-covid-19-frequently-asked-questions>, accessed 29 April 2020.
- [2] Tiner, S. "The Science Behind the Test for the COVID-19 Virus." *Discovery's Edge*. <https://discoverysedge.mayo.edu/2020/03/27/the-science-behind-the-test-for-the-covid-19-virus/>, 27 March 2020.
- [3] Bell, J. "Covid-19 Testing Kits: What Are They, Who Makes Them, and Why Are There Shortages?" NS Medical Devices, <https://www.nsmmedicaldevices.com/news/covid-19-testing-kits-shortages/>, 17 April 2020.
- [4] Jawerth, N. "How Is the COVID-19 Virus Detected Using Real Time RT-PCR?" International Atomic Energy Agency, <https://www.iaea.org/newscenter/news/how-is-the-covid-19-virus-detected-using-real-time-rt-pcr>, 27 March 2020.
- [5] Abbott. "Abbott RealTime SARS-CoV-2." FDA, <https://www.fda.gov/media/136258/download>, March 2020.
- [6] U.S. Department of Homeland Security. Personal communication, 28 April 2020.
- [7] BD. "BD, BioMedomics Announce Launch of Rapid Serology Test to Detect Exposure to COVID-19." <https://www.bd.com/en-us/company/news-and-media/press-releases/bd-biomedomics-announce-launch-of-rapid-serology-test-to-detect-exposure-to-covid-19>, 31 March 2020.
- [8] ChemBio Diagnostic Systems, Inc. "DPP COVID-19 IgM/IgG System." FDA, <https://www.fda.gov/media/136963/download>, accessed 24 April 2020.
- [9] Japsen, B. "Abbott Labs Rolling Out Coronavirus Antibody Tests." *Forbes*, <https://www.forbes.com/sites/brucejapsen/2020/04/15/abbott-labs-rolling-out-coronavirus-antibody-tests/#6da2d64a798d>, 15 April 2020.
- [10] Abbott. "SARS-CoV-2 IgG." FDA, <https://www.fda.gov/media/137383/download>, April 2020.
- [11] Abbott. "Detect COVID-19 in as Little as 5 Minutes." <https://www.abbott.com/corpnewsroom/product-and-innovation/detect-covid-19-in-as-little-as-5-minutes.html>, 27 March 2020.
- [12] FDA. "ID NOW COVID-19 - Letter of Authorization." <https://www.fda.gov/media/136522/download>, 27 March 2020.

- [13] ThermoFisher Scientific. "TaqPath COVID-19 Multiplex Diagnostic Solution." <https://www.thermofisher.com/us/en/home/clinical/clinical-genomics/pathogen-detection-solutions/coronavirus-2019-ncov/genetic-analysis/tagpath-rt-pcr-covid-19-kit.html>, accessed 27 April 2020.
- [14] FDA. "Thermo Fisher LOA EUA." <https://www.fda.gov/media/136113/download>, 13 March 2020.
- [15] Roche. "cobas® SARS-CoV-2." FDA, <https://www.fda.gov/media/136049/download>, accessed 4 May 2020.
- [16] Roche. "Roche's cobas SARS-CoV-2 Test to Detect Novel Coronavirus Receives FDA Emergency Use Authorization and Is Available in Markets Accepting the CE Mark." <https://www.roche.com/media/releases/med-cor-2020-03-13.htm>, 13 March 2020.
- [17] Roche. "Roche COVID-19 (Coronavirus) Updates." <https://diagnostics.roche.com/us/en/landing-pages/roche-covid-19-updates.html>, accessed 28 April 2020.
- [18] Siemens Fast Track Diagnostics. "Respiratory Infections." <http://www.fast-trackdiagnostics.com/media/1151032/FTD-SARS-CoV-2-Assay-RUO-Spec-Sheet-0420-FINAL.pdf>, April 2020.
- [19] Biomerieux. "NUCLISENS easyMAG." <https://www.biomerieux-usa.com/clinical/nuclisens-easymag>, accessed 29 April 2020.
- [20] Winter, L. "First Saliva Test for COVID-19 Approved for Emergency Use by FDA." The Scientist, <https://www.the-scientist.com/news-opinion/first-saliva-test-for-covid-19-approved-for-emergency-use-by-fda-67416>, 14 April 2020.
- [21] Greenwood, M. "Saliva Samples Preferable to Deep Nasal Swabs for Testing COVID-19." *YaleNews*, <https://news.yale.edu/2020/04/24/saliva-samples-preferable-deep-nasal-swabs-testing-covid-19>, 24 April 2020.
- [22] Wyllie, A. L., et al. "Saliva Is More Sensitive for SARS-CoV-2 Detection in COVID-19 Patients Than Nasopharyngeal Swabs." *MedRxiv*, <https://www.medrxiv.org/content/10.1101/2020.04.16.20067835v1>, 22 April 2020.
- [23] FDA. "Accelerated Emergency Use Authorization (EUA) Summary SARS-CoV-2 Assay (Rutgers Clinical Genomics Laboratory)." <https://www.fda.gov/media/136875/download>, 10 April 2020.
- [24] Karow, J. "Alternative Sample Types Could Boost COVID-19 Testing." *Modern Healthcare*, <https://www.modernhealthcare.com/patients/alternative-sample-types-could-boost-covid-19-testing>, 30 April 2020.

- [25] Centers for Disease Control and Prevention. "COVID-19 Testing and Reporting by Laboratories: Q & A." <https://www.cdc.gov/coronavirus/2019-ncov/lab/testing-laboratories.html>, accessed 4 May 2020.
- [26] Federal Emergency Management Agency. "Coronavirus (COVID-19) Pandemic: International Reagent Resource." <https://www.fema.gov/news-release/2020/04/13/coronavirus-covid-19-pandemic-international-reagent-resource>, 13 April 2020.
- [27] Millipore Sigma. "Coronavirus COVID-19 (SARS-CoV-2) Detection, Characterization, Vaccine and Therapy Production." https://www.sigmaaldrich.com/covid-19.html?cm_sp=JustUno--COVID19--Awareness, accessed 28 April 2020.
- [28] Venture Radar. "Similar Companies to Sigma-Aldrich." <https://www.ventureradar.com/similar/Sigma-Aldrich/ba6efb9c-872a-47b2-afba-ae8e2f40947d>, accessed 28 April 2020.
- [29] Kelley, G., and K. Ward. "MSU Researchers Identify New COVID-19 Testing Process." MSU Today, <https://msutoday.msu.edu/news/2020/msu-researchers-identify-new-covid-19-testing-process/>, 16 April 2020.
- [30] Mehta, A. "Mystery Surrounds UK Claim of COVID-19 Test Reagent 'Shortage'." Chemistry World, <https://www.chemistryworld.com/news/mystery-surrounds-uk-claim-of-covid-19-test-reagent-shortage/4011457.article>, 3 April 2020.
- [31] Chien, A., D. B. Edgar, and J. M. Trela. "Deoxyribonucleic Acid Polymerase From the Extreme Thermophile *Thermus Aquaticus*." *Journal of Bacteriology*, vol. 127, no. 3, pp. 1550–1557, <https://pubmed.ncbi.nlm.nih.gov/8432/>, September 1976.

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