

TITLE: ASSAY TEST DEVICE AND METHOD

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DEVELOPMENT**

Not Applicable.

FIELD OF THE INVENTION

This invention relates to medical assay tests, and more particularly to a combination
assay test for detecting medical conditions.

BACKGROUND

Rapid diagnostic tests have become increasingly important in modern healthcare, offering
quick and accessible means of detecting various medical conditions. These tests are
particularly valuable in point-of-care settings and for home use, where immediate results
can guide timely medical decisions.

1 Traditional rapid tests often focus on detecting a single biomarker, which can limit their
2 diagnostic accuracy and reliability. Additionally, many existing rapid test kits consist of
3 multiple separate components, making them complex to use and prone to user error,
4 especially for untrained individuals performing self-tests.

5
6 There is a growing need for rapid diagnostic devices that can detect multiple biomarkers
7 simultaneously, thereby increasing diagnostic accuracy and expanding the range of
8 conditions that can be screened. Such multi-biomarker tests could provide more
9 comprehensive health information and potentially reduce the need for multiple separate
10 tests.

11
12 Furthermore, the usability of rapid tests remains a significant challenge. Complex multi-
13 step procedures and the need to handle multiple components can lead to errors in sample
14 collection, application, and result interpretation. These issues are particularly problematic
15 in self-testing scenarios or in resource-limited settings where professional healthcare
16 support may not be readily available.

17
18 The integration of digital technologies with rapid tests also presents opportunities for
19 improving result interpretation, data management, and remote healthcare support.
20 However, many current rapid test designs are not optimized for such digital integration.

21
22 There exists a need for an improved rapid diagnostic device that can detect multiple
23 biomarkers simultaneously, offer enhanced ease of use, reduce the potential for user

error, and be compatible with digital health technologies. Such a device could significantly advance the field of point-of-care and home-based diagnostics, potentially improving health outcomes through earlier and more accurate detection of various medical conditions. The present invention accomplishes these objectives.

SUMMARY OF THE INVENTION

The present invention provides a rapid diagnostic device for detecting multiple biomarkers in a bodily fluid sample. The device comprises a housing containing at least one test strip, each test strip having a sample receiving zone, at least one conjugate pad, multiple test zones, and a control zone. The conjugate pad or pads together contain dried particle conjugates specific to at least two distinct biomarkers, allowing for simultaneous detection of multiple biomarkers on a single test strip.

The device is designed to detect at least two different biomarkers, which may include combinations such as hCG and a cancer antigen selected from CA125, CA15-3, CA19-9, CA27.29, or CA549. This multi-biomarker approach enhances the accuracy and reliability of diagnoses for specific medical conditions.

Key features of the device include its compatibility with various sample types (whole blood, serum, plasma, and urine), an integrated buffer system for consistent sample preparation, and a built-in desiccant to maintain optimal humidity levels. The device is

1 engineered to provide rapid results, typically within ten minutes or less after sample
2 application.

3
4 Additional features may include a built-in safety lancet for blood sample collection,
5 compatibility with point-of-care readers for quantitative analysis, and integration with
6 mobile applications for result interpretation and data management. The housing is
7 designed with a viewing window aligned with the test and control zones, allowing for
8 easy result visualization.

9
10 This innovative rapid diagnostic device offers a user-friendly, efficient, and versatile
11 solution for point-of-care testing and home use, potentially improving early detection and
12 monitoring of various medical conditions. The present invention overcomes the
13 limitations of prior art devices by providing an improved rapid diagnostic device capable
14 of detecting multiple biomarkers simultaneously. This innovative device offers enhanced
15 ease of use through its integrated design, significantly reducing the potential for user
16 error. The invention incorporates features that make it compatible with digital health
17 technologies, allowing for improved result interpretation, data management, and remote
18 healthcare support. Other features and advantages of the present invention will become
19 apparent from the following more detailed description, taken in conjunction with the
20 accompanying drawings, which illustrate, by way of example, the principles of the
21 invention.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is an exploded perspective view of one embodiment of the invention, illustrating a first embodiment having a single test strip with multiple test zones;

FIG. 2 is a top plan view of a test strip of an alternate embodiment, wherein a portion of the test strip is split into two separate conjugate pads and test zones;

FIG. 3 is a top plan view of a test strip of another alternate embodiment having two parallel and adjacent test strips; and

FIG. 4 is a top plan view of a test strip of yet another alternate embodiment, similar to the embodiment of FIG. 2 but having a liquid impervious barrier between the conjugate pads and the test zones.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Illustrative embodiments of the invention are described below. The following explanation provides specific details for a thorough understanding of and enabling description for these embodiments. One skilled in the art will understand that the invention may be practiced without such details. In other instances, well-known structures and functions have not been shown or described in detail to avoid unnecessarily obscuring the description of the embodiments.

1 Unless the context clearly requires otherwise, throughout the description and the claims,
2 the words "comprise," "comprising," and the like are to be construed in an inclusive sense
3 as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including,
4 but not limited to." Words using the singular or plural number also include the plural or
5 singular number respectively. Additionally, the words "herein," "above," "below" and
6 words of similar import, when used in this application, shall refer to this application as a
7 whole and not to any particular portions of this application. When the claims use the
8 word "or" in reference to a list of two or more items, that word covers all of the following
9 interpretations of the word: any of the items in the list, all of the items in the list and any
10 combination of the items in the list. When the word "each" is used to refer to an element
11 that was previously introduced as being at least one in number, the word "each" does not
12 necessarily imply a plurality of the elements, but can also mean a singular element.

13
14 FIGS. 1 and 2 illustrate a rapid diagnostic device 10 for detecting multiple biomarkers in
15 a bodily fluid sample 20. The rapid diagnostic device 10 comprises a housing 30 that
16 encloses and protects internal components. The housing 30 may be made of plastic,
17 biodegradable plastic, recycled materials, or the like.

18
19 At least one test strip 40, and in some embodiments, two or more test strips 40, are
20 disposed within the housing 30, as shown in FIG. 2. When multiple test strips 40 are
21 present, they may be arranged substantially parallel to each other and mutually adjacent
22 (FIG. 3). Each test strip 40 comprises a sample receiving zone 50 at the first end 41

1 configured to receive the bodily fluid sample 20. The sample receiving zone 50 may be
2 made of materials such as cellulose, glass fiber, polyester, or the like.

3
4 Adjacent to the sample receiving zone 50 is at least one conjugate pad 60 containing
5 dried particle conjugates 70 specific to at least two distinct biomarkers. In some
6 embodiments, there may be two or more conjugate pads 60, each containing dried
7 particle conjugates 70 specific to one of the distinct biomarkers. The conjugate pad 60
8 may be composed of materials such as glass fiber, polyester, or the like. The dried
9 particle conjugates 70 may comprise colloidal gold nanoparticles, colored latex particles,
10 fluorescent particles, or the like.

11
12 Each test strip 40 further includes at least two test zones 80 containing immobilized
13 capture reagents 90 for detecting the distinct biomarkers, and a control zone 100 for
14 indicating proper test function. The test zones 80 and control zone 100 may be composed
15 of nitrocellulose, nylon, or the like. The immobilized capture reagents 90 may include
16 antibodies, antigens, aptamers, or the like, specific to the biomarker being detected. In
17 some embodiments, the test zones 80 are mutually adjacent.

18
19 The concentration of dried particle conjugates 70 for hCG and CA-15-3 is crucial for
20 achieving optimal test sensitivity and specificity. For hCG detection, the conjugate
21 concentration may be optimized to detect levels as low as 5-10 mIU/ml, which is
22 typically indicative of early pregnancy or certain pathological conditions. In the case of
23 CA-15-3, the conjugate concentration should be calibrated to detect levels above 30

1 U/ml, which is generally considered the upper limit of the normal range and may indicate
2 the presence of breast cancer or other malignancies. However, it's important to note that
3 the exact concentrations may vary depending on the specific antibodies used and the
4 desired test performance characteristics. For instance, in some embodiments, the hCG
5 conjugate may be adjusted to detect levels between 0.1 mU/ml and 4 mU/ml to
6 differentiate between benign and malignant ovarian tumors, as studies have shown
7 significant differences in hCG serum concentrations at these levels. Similarly, for CA-15-
8 3, the conjugate concentration may be fine-tuned to detect levels around 40 U/ml, which
9 has been associated with poor prognosis in some cancer patients. For each embodiment of
10 the invention, the optimal conjugate concentrations are determined through extensive
11 testing and validation to ensure the rapid diagnostic device 10 provides reliable results
12 across a clinically relevant range of biomarker concentrations.

13
14 The housing 30 includes an opening 31 at a first end 33 thereof that opens to the first end
15 41 of each test strip 40. The opening 31 allows for introduction of the bodily fluid sample
16 20 into the device 10. The housing 30 also includes a viewing window 32 aligned with
17 the test zones 80 and the control zone 100 of each test strip 40, allowing for visual
18 inspection of test results.

19
20 The bodily fluid sample 20 flows along each test strip 40 from the sample receiving zone
21 50 through the at least one conjugate pad 60 to the test zones 80 and control zone 100.
22 This flow may be facilitated by capillary action, ensuring proper sample distribution and
23 interaction with the reagents. Each test strip 40 is configured to detect at least two

1 different biomarkers, allowing for simultaneous detection of multiple biomarkers in a
2 single test enclosure. A positive result in all test zones 80 indicates an overall positive test
3 result for a specific medical condition.

4
5 In one embodiment, the distinct biomarkers include hCG and one of the cancer antigens
6 taken from the group consisting of: CA125, CA15-3, CA19-9, CA27.29, and CA549.
7 This combination allows for simultaneous detection of these distinct biomarkers, which
8 may be indicative of certain medical conditions when present together, such as various
9 types of cancer. For example, testing for hCG and CA15-3 can help detect breast
10 cancers.

11
12 The rapid diagnostic device 10 may further comprise a built-in safety lancet (not shown)
13 for collecting a blood sample. The safety lancet may be integrated into the housing 30
14 and designed to retract after use to prevent accidental injury. This feature allows for
15 convenient and safe sample collection, particularly for home use or point-of-care testing.

16
17 In some embodiments, the sample receiving zone 50 includes an integrated buffer 120 for
18 delivering a predetermined volume of buffer to the at least one test strip 40. This
19 integrated buffer system ensures consistent and appropriate buffer application, which is
20 crucial for test accuracy.

21
22 The rapid diagnostic device 10 may be compatible with a point-of-care reader (not
23 shown) for quantitative or semi-quantitative analysis of test results. The reader may use

1 optical, electrochemical, or other detection methods to provide more precise results than
2 visual interpretation alone.

3
4 The rapid diagnostic device 10 may also be used in conjunction with a mobile application
5 for capturing and analyzing test results using a smartphone camera (not shown). The
6 application may provide step-by-step instructions, timer functions, result interpretation,
7 and data storage capabilities.

8
9 In some embodiments, where the bodily fluid sample 20 is whole blood, the device may
10 further comprise a blood separation membrane 150 for separating plasma or serum from
11 the whole blood sample. The membrane 150 may be placed before the sample receiving
12 zone 50 and may be made of materials such as asymmetric polysulfone, glass fiber, or the
13 like. In some embodiments wherein the dried particle conjugates of various
14 combinations can detrimentally interact, a liquid impervious barrier 190 (FIG. 4) is
15 disposed between the two or more conjugate pads 60 and the test zones 80, whereby
16 cross-contamination between conjugate pads 60 and the test zones 80 is inhibited.

17
18 The rapid diagnostic device 10 may be configured to be compatible with multiple sample
19 types including whole blood, serum, plasma, and urine. This versatility allows for broader
20 application and user convenience.

1 To maintain the integrity of the test strips 40 and reagents, the rapid diagnostic device 10
2 may include a desiccant 160 (FIG. 1) within the housing 30 to maintain low humidity
3 conditions. The desiccant 160 may be silica gel, molecular sieves, or the like.

4
5 The rapid diagnostic device 10 is designed to provide results preferably within ten
6 minutes or less after sample application, allowing for quick diagnosis and decision-
7 making. This rapid result time, combined with the multi-biomarker detection capability,
8 makes the device particularly suitable for point-of-care testing and home use scenarios.

9
10 The rapid diagnostic device 10 also may include a backing card 170 and an absorbent pad
11 180. The backing card 170 serves as a support structure for the entire test strip (40). It
12 may be made of materials such as polyvinyl chloride (PVC), polystyrene, or other rigid,
13 non-porous plastics. The backing card 170 provides structural integrity to the device 10
14 and helps maintain the proper alignment of all components. In some embodiments, the
15 backing card 170 may be coated with an adhesive to securely hold the sample pad,
16 conjugate pad(s), nitrocellulose membrane test zone(s) 80, and absorbent pad 180 in
17 place. The choice of adhesive is important, as it must not interfere with the biological
18 components of the test or degrade over time. The thickness of the backing card 170 may
19 vary depending on the specific application, with thinner materials often used for cassette-
20 based tests and thicker materials for dipstick-style tests.

21
22 The absorbent pad 180, also known as the wick pad, is typically placed at the end of the
23 test strip 40 opposite to the sample receiving zone 50. It is usually made of cellulose

1 fibers or other highly absorbent materials. The primary function of the absorbent pad 180
2 is to draw the sample and reagents through the test strip by capillary action, ensuring
3 proper flow and preventing backflow. The absorbent capacity of the pad 180 is important,
4 as it must be able to hold the entire volume of sample and buffer used in the test.
5 Different grades of absorbent materials may be chosen based on their wicking rates to
6 control the overall flow rate of the assay.

7
8 The buffer solution used in the rapid diagnostic device 10 plays an important role in the
9 performance and reliability of the test. The buffer typically contains several components,
10 each serving a specific purpose:

- 11 1. pH buffer: This maintains the optimal pH for antibody-antigen interactions and
12 enzyme activity. Common pH buffers include phosphate, Tris, and borate.
- 13 2. Surfactants: These help in sample flow and reduce non-specific binding. Examples
14 include Tween 20, Triton X-100, and SDS.
- 15 3. Proteins: Proteins such as BSA (Bovine Serum Albumin) or casein may be added to
16 block non-specific binding sites and stabilize other proteins in the system.
- 17 4. Preservatives: These prevent microbial growth in the buffer. Common preservatives
18 include sodium azide and ProClin.
- 19 5. Salts: Salts like sodium chloride help maintain the ionic strength of the solution,
20 which is important for proper antibody-antigen interactions.

1 The composition of the buffer may be optimized for each specific assay to ensure
2 maximum sensitivity and specificity. The ionic strength, pH, and concentrations of
3 various components are carefully balanced to achieve optimal test performance.
4

5 The particle conjugates 70 specific to at least two distinct biomarkers are a key feature of
6 this rapid diagnostic device 10. These conjugates are typically prepared by attaching
7 antibodies or antigens specific to the target biomarkers onto the surface of colloidal gold
8 particles, colored latex beads, or fluorescent particles. The size of these particles is
9 important for optimal performance, with 40nm being a common size for colloidal gold
10 particles. The conjugation process must be carefully controlled to ensure that the
11 biological activity of the antibodies or antigens is preserved while achieving stable
12 attachment to the particles.
13

14 By incorporating these various components and carefully optimizing their interactions,
15 the rapid diagnostic device 10 achieves high sensitivity, specificity, and reliability in
16 detecting multiple biomarkers simultaneously, providing a powerful tool for point-of-care
17 diagnostics and health monitoring.
18

19 While the embodiment described above features at least one test strip 40 for detecting
20 multiple biomarkers, it should be understood that the device may incorporate multiple of
21 the particle conjugates (70) specific to three, four, or more distinct biomarkers for various
22 diagnostic applications. The arrangement of the test strip(s) 40 within the housing 30

ensures efficient use of space and allows for simultaneous testing of multiple biomarkers using a single sample, and simultaneous viewing of the combined test results.

In embodiments with two or more conjugate pads 60, a liquid impervious barrier may be disposed between the conjugate pads 60 and the test zones 80. This barrier helps to inhibit cross-contamination between conjugate pads 60 and the test zones 80 for applications where cross-contamination can jeopardize test results, ensuring the accuracy and reliability of the test results.

The number and type of test strips 40 may be selected based on the specific medical condition or set of conditions being screened for. For example, in addition to hCG and cancer antigens, the device may include test strips for detecting other cancer biomarkers, hormones, infectious disease markers, or other clinically relevant analytes. This flexibility in the number and type of test strips 40 allows the rapid diagnostic device 10 to be adapted for various screening and diagnostic needs.

A key feature of the rapid diagnostic device 10 is that a positive result on all test strips indicates an overall positive test result for a specific medical condition. This multi-biomarker approach can increase the accuracy and reliability of the diagnosis compared to single-marker tests. For instance, in the case of the hCG and cancer antigen combination, the simultaneous detection of elevated levels of both markers may provide a stronger indication of a particular type of cancer than either marker alone. The

1 interpretation of results and the specific medical conditions indicated by positive results
2 on all strips may vary depending on the combination of biomarkers chosen for the device.
3

4 While a particular form of the invention has been illustrated and described, it will be
5 apparent that various modifications can be made without departing from the spirit and
6 scope of the invention. Accordingly, it is not intended that the invention be limited,
7 except as by the appended claims.
8
9

1 **CLAIMS**

2
3 What is claimed is:

4
5 1. A rapid diagnostic device for detecting multiple biomarkers in a bodily fluid sample,
6 comprising:

7 a housing;

8 at least one test strip disposed within the housing, each test strip comprising:

9 a sample receiving zone at a first end configured to receive the bodily fluid

10 sample,

11 at least one conjugate pad containing dried particle conjugates specific to at least

12 two distinct biomarkers,

13 at least two test zones each containing immobilized capture reagents for detecting

14 one of the distinct biomarkers, and

15 a control zone for indicating proper test function;

16 wherein the bodily fluid sample flows along each test strip from the sample

17 receiving zone through the at least one conjugate pad to the test zones and control zone;

18 wherein the at least one test strip is configured to detect at least two different

19 biomarkers; and

20 wherein a positive result in all test zones indicates an overall positive test result

21 for a specific medical condition.

22

2. A rapid diagnostic device for detecting multiple biomarkers in a bodily fluid sample,
comprising:
a housing made of a material selected from the group consisting of plastic,
biodegradable plastic, and recycled materials;
two or more test strips disposed substantially parallel within the housing, each test
strip comprising:
a sample receiving zone at a first end configured to receive the bodily fluid
sample, wherein the sample receiving zone includes an integrated buffer for delivering a
predetermined volume of buffer to the test strip,
two or more conjugate pads, each containing dried particle conjugates specific to
one of at least two distinct biomarkers,
at least two mutually adjacent test zones each containing immobilized capture
reagents for detecting one of the distinct biomarkers, and
a control zone for indicating proper test function;
wherein the housing includes an opening at a first end thereof that opens to the first end
of the test strips;
wherein the housing includes a viewing window aligned with the test zones and
the control zone of the test strips;
wherein the bodily fluid sample flows along each test strip from the sample
receiving zone through the conjugate pads to the test zones and control zone;
wherein the test strips are configured to detect at least two different biomarkers,
include hCG and one of the cancer antigens taken from the group consisting of: CA125,
CA15-3, CA19-9, CA27.29, and CA549;

1 wherein a positive result in all test zones indicates an overall positive test result
2 for a specific medical condition;

3 wherein the device is configured to be compatible with multiple sample types
4 selected from the group consisting of whole blood, serum, plasma, and urine;
5 a desiccant within the housing to maintain low humidity conditions for the test strips; and

6 wherein the device is configured to provide results within ten minutes or less after
7 sample application.

8
9 3. A method for detecting multiple biomarkers in a bodily fluid sample, comprising:

10 providing a rapid diagnostic device comprising: a housing with an opening at a
11 first end and a viewing window, at least one test strip disposed within the housing, each
12 test strip comprising a sample receiving zone, at least one conjugate pad with dried
13 particle conjugates specific to at least two distinct biomarkers, at least two test zones with
14 immobilized capture reagents, and a control zone;

15 collecting a bodily fluid sample;

16 introducing the bodily fluid sample into the opening of the housing, thereby
17 contacting the sample receiving zone of the at least one test strip;

18 allowing the bodily fluid sample to flow along the at least one test strip from the
19 sample receiving zone through the at least one conjugate pad to the test zones and control
20 zone;

21 waiting for a predetermined time period not exceeding ten minutes;

22 observing the test zones and control zone of the at least one test strip through the
23 viewing window;

1 determining a test result for each biomarker based on the presence or absence of
2 visible indicators in the respective test zones; and
3 determining an overall test result, wherein a positive result in all test zones
4 indicates an overall positive test result for a specific medical condition.
5
6

ABSTRACT

A rapid diagnostic device for detecting multiple biomarkers in a bodily fluid sample is disclosed. The device comprises a housing containing at least one test strip with a sample receiving zone, at least one conjugate pad, multiple test zones, and a control zone. The conjugate pad contains dried particle conjugates specific to at least two distinct biomarkers, enabling simultaneous detection of multiple biomarkers on a single test strip. The device may detect combinations such as hCG and a cancer antigen selected from CA125, CA15-3, CA19-9, CA27.29, or CA549. Compatible with various sample types, the device can feature an integrated buffer, a built-in desiccant, and provides results within ten minutes. Additional features may include a safety lancet, compatibility with point-of-care readers, and mobile application integration. The device offers a user-friendly, efficient solution for point-of-care testing and home use, potentially improving early detection and monitoring of various medical conditions.

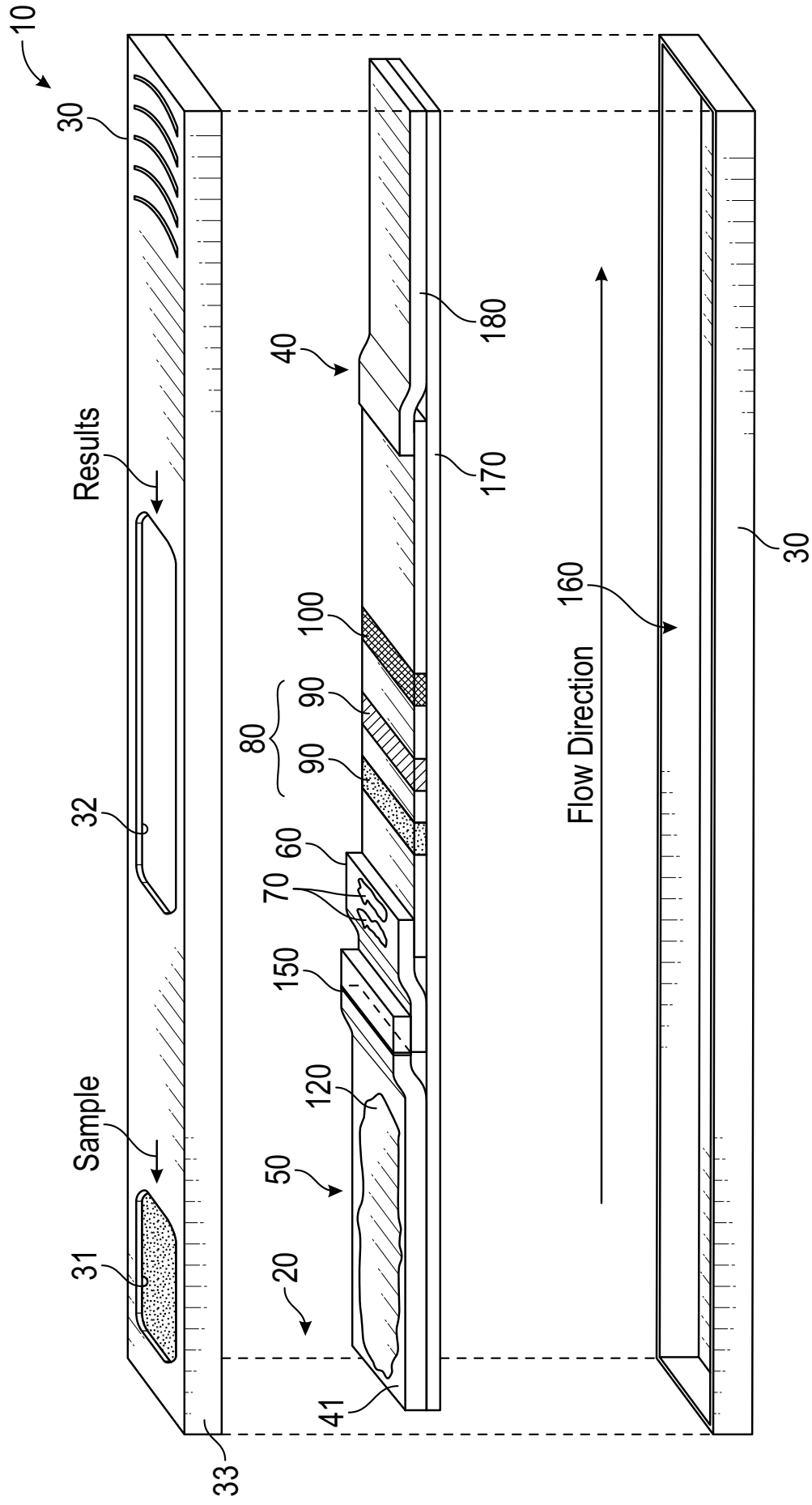


FIG. 1

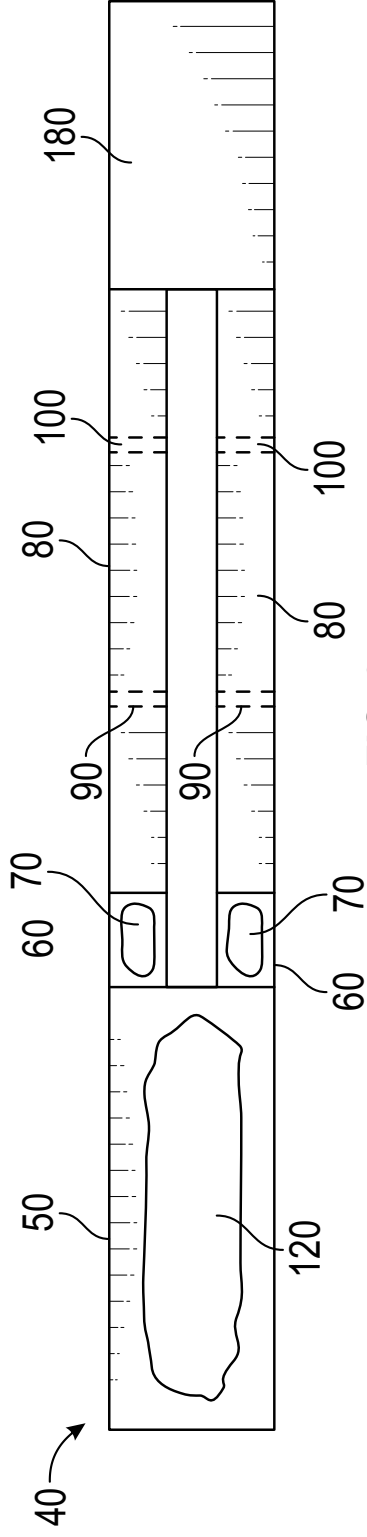


FIG. 2

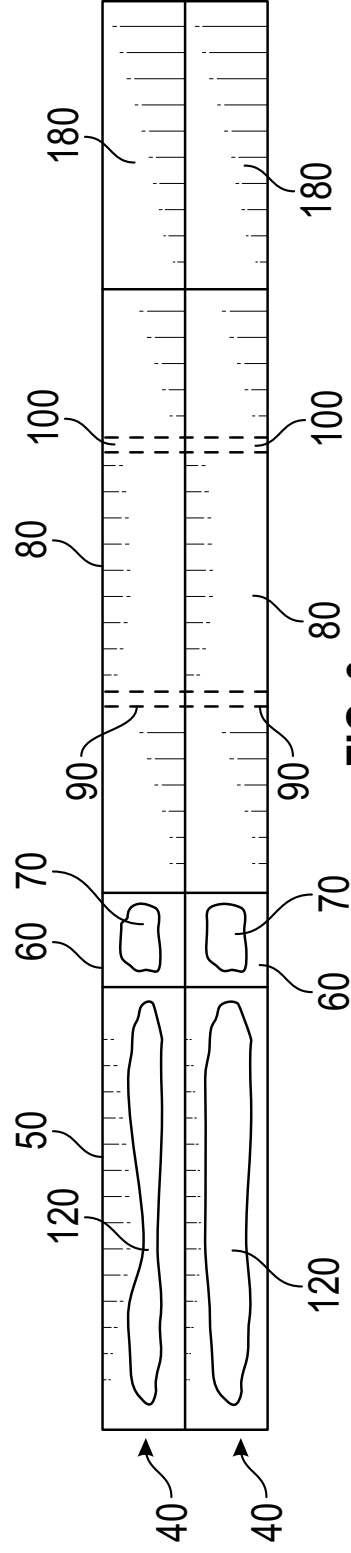


FIG. 3

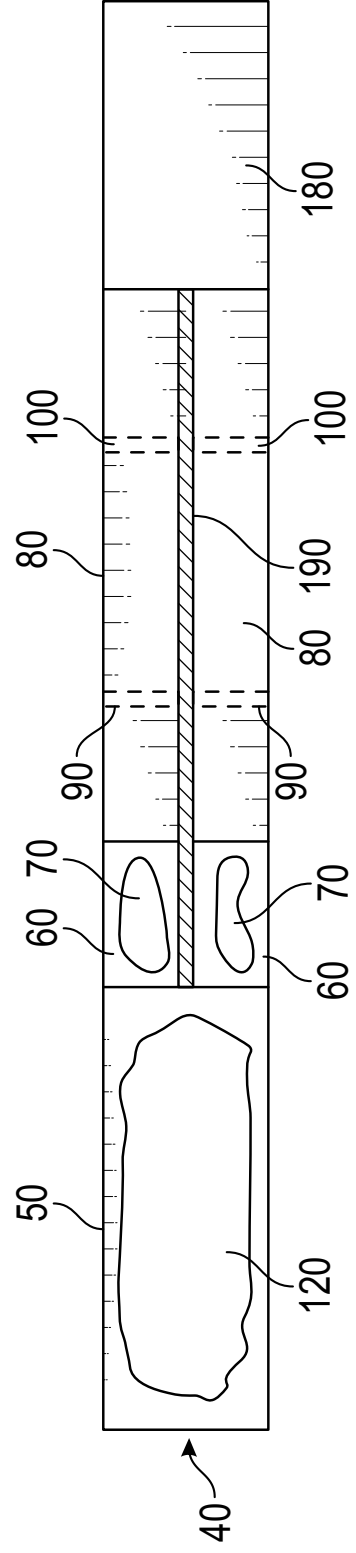


FIG. 4