A Modification of the “Sickledex” Test for Hemoglobin S

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The “Sickledex” test for hemoglobin S has been modified by the use of “Microtiter” plates and microliter sampling equipment. For screening purposes the testing procedure is faster, with significant glassware savings and a 10-fold decrease in sample and reagent requirements. Results are identical to those of the standard Sickledex test.

The “Sickledex” test (as described by Diggs et al., in a scientific exhibit, 1968, and marketed by Ortho Diagnostics, Raritan, N. J. 08869) has gained widespread use as a screening method for hemoglobin S (1, 2). Apparently based on the solubility test of Itano (3), it has been shown to be positive with abnormal hemoglobins other than the S hemoglobin (4). Nevertheless, it is a quick, simple screening test in which positive specimens can be further investigated by electrophoresis. This test has been shown to be more sensitive and more reliable than the standard metabisulfite sickle cell preparation (1).

We describe here a rapid method of performing the Sickledex test, with 10-fold less reagents and with further savings in time and glassware. This modification is intended to provide a more economical method for screening large populations.

Materials and Methods (2)

Apparatus

*Oxford Laboratory Samplers* (Oxford Laboratories, San Mateo, Calif. 94401) were used to pipet the Sickledex test solution in all instances, and the sample for the standard Sickledex test.

*A Hamilton syringe* (Hamilton Company, Whittier, Calif. 90608) was used to pipet the sample into the wells of the “Microtiter” plates.

*Microtiter plates* (Cooke Engineering Co., Alexandria, Va., 22314) that contain 96, U-shaped wells, replaced test tubes for the modified Sickledex test.

*“Agarose Film Cassettes System”* (Analytical Chemists, Inc., Palo Alto, Calif. 94303) was used for hemoglobin electrophoresis.

Reagents

*The Sickledex test solution* (Ortho) was prepared by adding the reagent powder to the diluent buffer according to kit instructions. The reagents and principles of this test have not been disclosed by the vendor. Conclusions as to the specificity and reliability can only be conjecture (4, 5).

*Sodium metabisulfite* (105 mmol/liter) was used for the standard sickle cell preparation and was prepared fresh daily by adding 200 mg to 10 ml distilled water.

Procedures

All blood specimens (EDTA anticoagulant) used in this study had hematocrits greater than 20. Standard sickle cell preparations were made by using freshly prepared sodium metabisulfite, and they were examined 30 min, 1 h, and 3 h later.

The standard Sickledex test was performed in 12 × 75 mm test tubes. To these, a 2.0-ml aliquot of Sickledex test solution was added, followed by 20 µl of the whole blood (EDTA anticoagulant). The contents were mixed and the results were read against a printed background after 5 min at room temperature. A cloudy, turbid suspension was graded as positive, a clear transparent suspension as negative. Positive and negative controls were processed with each run.

We prepared hemolysates of the whole blood specimens and electrophoresed them with the Agarose Film Cassettes System. Barbital buffer (pH 8.6, 50 mmol/liter) was used. The voltage gradient was 15 V/cm; electrophoresis was for 35 min.

We modified the Sickledex tests so we could use the plastic plates from the Microtiter System to perform the Sickledex test (Figure 1), and so decrease the volumes of reagent and blood needed by about 10-fold. A 250-µl aliquot of Sickledex test solution was added to each of the wells. The samples consisted of 2.5 µl of a whole blood hemolysate of about 10 g of hemoglobin/
plates
negative
ruled
perature.
by
blood
could
of
blood
was
re
formed-in
phoresis,
concentration.
and
sickling
hemoglobin
complete
agreement
among
the
various
methods
for
the
identification
of
the
hemoglobin
type.
One
specimen,
which
was
weakly
positive
for
hemoglobin
SA,
required
3
h
by
the
metabisulfite
sickle
preparation
to
come
positive.
The
same
specimen
was
more
weakly
positive
by
the
two
Sickledex
methods,
and
the
S
band
by
electrophoresis
was
smaller
than
in
the
other
positives.

Discussion

We
found
the
Sickledex
test
to
be
easily
adapted
to
the
Microtiter
plate,
with
no
loss
in
sensitivity.
The
plates
contain
96
wells
each
and
they
can
be
easily
and
quickly
filled
with
Sickledex
test
reagent
by
the
use
of
automatic
pipets,
of
which
many
sorts
are
available
commercially.
The
most
laborious
task
is
to
add
the
sample,
which
in
this
case
consists
of
2.5
μl
of
hemolsate.
We
used
the
Hamilton
syringe,
but
microcapillary
tubes
are
equitably
satisfactory.
Once
the
reagents
and
samples
are
added,
the
entire
plate
can
be
shaken
by
hand
on
a
flat
surface
with
a
rapid
oscillatory
motion.
Ninety-six
specimens
can
be
processed
and
interpreted
in
less
than
1.5
h.
Further
improvements
in
automatic
microsampling
and
reagent
delivery
are
being
investigated.

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for
supplying
blood
samples.
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assistance.

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of
tests
for
hemoglobin
S.
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Med.
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Pathol.
10,
277

Fig. 1. Microtiter
plate
with
11
positive
specimens
arranged
across
the
top
on
the
first
row
(the
right-hand
column
is
empty).
The
remaining
77
specimens
are
negative
for
sickling
hemoglobin
100
ml
concentration.
We
have
found
that
either
whole
blood
or
a
whole
blood
hemolysate
can
be
used.
The
plates
were
covered
with
Parafilm,
vigorously
shaken
by
hand,
and
allowed
to
stand
for
5
min
at
room
temperature.
The
plates
were
then
placed
derover
a
heavily
ruled
translucent
background;
in
the
positive
tests
it
was
difficult
or
impossible
to
see
the
lines,
while
negative
specimens
were
transparent
and
the
lines
could
be
easily
seen.
A
viewbox,
of
the
type
used
in
blood
banks,
facilitated
the
reading
and
interpretation
of
the
plates.

Eighty-eight
specimens
were
investigated.
Their
origin
was
not
identified
as
to
age,
sex,
or
race
of
the
patient,
and
several
of
the
specimens
had
been
previously
identified
as
abnormal.
A
sickle
cell
preparation,
a
standard
Sickledex
test,
hemoglobin
electrophoresis,
and
our
modified
Sickledex
tests
were
performed—in
that
order—on
each
sample.
The
samples
were
refrigerated
when
not
being
actually
tested.

Results

Of
the
88
specimens,
four
were
classified
as
hemoglobin
SA,
seven
as
hemoglobin
SS,
and
the
rest
as
normal.
There
was
complete
agreement
among
the